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Title/Titre
Seed Potato Certification Program - Requirements for the production of Pre-Elite seed potatoes from sources other than Nuclear Stock.

Our File/Notre référence
3700-2-1

I. SUBJECT

This directive contains the requirements for the production of Pre-Elite seed potatoes from material other than **nuclear stock**[1], i.e. **disease free** cuttings, plants, tubers, or **selected clones** (*Seeds Regulations C.R.C. c. 1400*, section 47.2). In this directive, the term **nuclear stock equivalent** will be used for this material.

This directive supersedes all previous drafts of this subject.

II. BACKGROUND

Although there has been a general movement toward a flush through system starting with disease tested plantlets grown in vitro (**nuclear stock**), there is still some interest in using other sources of seed to start new field production of seed potatoes. For comparison, Holland, the number one seed potato exporting country, is extensively using **clonal selection** in its own seed certification system. Therefore, the need has been identified to maintain the possibility of doing clonal selection in Canada. The present directive does specify the criteria that have to be met in order to produce Pre-Elite seed potatoes from material other than **nuclear stock**.

[1] See section V. for a definition of words appearing in bold in the text.

III. LEGISLATIVE AUTHORITY

Seeds Act R.S., c. S-8 and amendments 1976-77, c.28 and 1985, c.47.
Seed Regulations and their amendments, SOR/91-526, SOR/93-331, SOR/95-179, SOR/95-215 and SOR/97-118.

IV. POLICY

Any grower intending to use **nuclear stock equivalent** to produce Pre-Elite seed potatoes must comply with the following requirements (flowchart in Appendix 1):

1. SELECTION AND HARVEST

Mother plants must be selected from a field certified by the Canadian Food Inspection Agency (CFIA) under the Seed Potato Certification program. **Selected clones** are specifically chosen because they express characteristics such as freedom from visual symptoms of disease and physiological defects, uniform size and set of tubers, and varietal trueness to type.

At this point, field testing of the **mother plants** to eliminate material that could be infected by viruses is highly recommended but not required. Because this will permit elimination of plants affected by **secondary infection**, but probably only a part of those affected by **primary infection**, a post-harvest test is required anyway.

Growers or provincial specialists are responsible for the selection of clones. It is not the CFIA responsibility to do so.

Handling of the **selected clones** is the responsibility of the grower or provincial specialist, and will be monitored and audited by CFIA.

Each clone must be harvested, labelled and bagged separately.

2. TESTING

As mentioned before, post-harvest testing is mandatory to insure the elimination of plants affected by **secondary infection**.

Because of the uneven distribution of diseases over the tubers of a **selected clone**, and because low concentrations of a disease causing organism can be undetectable by laboratory testing, all tubers (*or their progeny*) from the selected **clone**(s) must be post-harvest tested, even if

not all tubers are intended to be replanted to produce Pre-Elite seeds.

All required testing (bacterial ring rot, PSTVd, and viruses) must be done at growers' expense in a CFIA accredited laboratory.

2.1 Bacterial Ring Rot (BRR) testing

In the case of BRR, testing must be done directly on the tubers harvested from the **selected clones**. Stem testing in the greenhouse or in the field the next season, or tuber testing in the greenhouse, are not considered acceptable alternatives; indeed, an equivalent test would require every single stem or tuber to be tested.

Core samples must be taken from the stem end of each tuber harvested from the **mother plant**, and tested for *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff 1914) Davis, Gillaspies, Vidaver & Harris 1984, the causal agent of bacterial ring rot (see Appendix 2 for sampling details).

If any core sample tests positive for bacterial ring rot, all **clones** from the same farm unit will be rejected for the production of Pre-Elite seeds. All lots from that farm unit will be decertified, and standard procedures related to bacterial ring rot infection will apply.

2.2 Virus and viroid testing

Virus and viroid testing can be carried out in two ways: in the greenhouse during the winter (see section 2.2.1 for details), or in the field the spring following harvest (see section 2.2.2 for details).

If any sample tests positive for any viruses, the entire **clone** must be rejected for the production of Pre-Elite seeds. Positive **clones** may still be eligible for certification, provided all regulatory requirements are met.

If any sample test positive for the presence of PSTVd, the entire seed lot from which the clone originates will be decertified as outlined in section 52.(5)(e) of the *Seeds Regulations*.

When testing is done in the field (as in section 2.2.2), all positive **clones** must be removed from the field for certification at the Pre-Elite class. If not, the entire field will be downgraded to a lower class, provided all regulatory requirements are met.

2.2.1 Virus and viroid testing in the greenhouse during the winter

(alternative 1)

One eye from each tuber in the **clone** can be sprouted and/or grown out. When removing the eye appropriate care has to be taken to avoid any possibilities of cross contamination between **clones**. To reduce the risk of contamination it is recommended to disinfect knives and equipment between each tuber. Leaf or sprout tissues produced from each excised eye must be tested for PLRV, PSTVd, PVA, PVM, PVS, PVX, PVY^{o}, PVY^{n} and RLSV (see Appendix 3 for details). **Clones** of the same variety that test negative for all diseases can be planted in bulk, and do not need to be kept separate, as no further testing is required.

2.2.2 Virus and viroid testing in the field, the spring following harvest of selected clones (alternative 2)

After planting of the **selected clones** the spring following harvest, terminal three leaflets from fully expanded young compound leaves are taken from at least one plant from each **tuber unit** and tested for PLRV, PSTVd, PVA, PVM, PVS, PVX, PVY^{o}, PVY^{n}, and RLSV (see appendix 3 for sampling details). Each **clone** must be properly identified and planted separately in the field. **Clones** must be planted so that no contact is possible between them before positive ones can be removed. Cut seed must be planted as tuber units, and spacing between each unit is required.

3. FURTHER MULTIPLICATION

When tubers are tested (as in section 2.2.1) and are confirmed to be **disease free**, further multiplication is allowed before planting in the field. Cuttings must be produced in a **protected environment**. No further testing is required for these cuttings. If the process of multiplication started before all required testing is completed, care must be taken to avoid any possible cross contamination between clones and every clone must be kept separate and clearly identified.

Plants that are field tested (as in section 2.2.2) may only be planted as whole seed or be multiplied by cutting the tubers. Extra care must be taken, if tubers are being cut, to avoid cross contamination between clones. To reduce the risk of contamination it is recommended to disinfect knives and equipment between each tuber.

4. APPLICATION FOR CERTIFICATION

Application for seed potato crop inspection and field inspections will be the same as for the production of Pre-Elite from **nuclear stock**. At the time of first inspection or earlier the applicant for crop inspection must show to the satisfaction of the inspector that all the testing has been completed. Documentation on **clonal selection**, clone identification, testing results etc... must be made available to the inspector anytime.

V. DEFINITIONS

Clone: all tubers and progeny of one **mother plant**.

Clonal selection: process by which field grown tubers are used to initiate a new line of seed potatoes.

Disease free: seed potatoes tested following **official protocol** and found negative on all the tests.

Mother plant: any individual plant, or plant part that will be used to start a multiplication process; the progeny of such entity forms a **clone**.

Nuclear stock: Refer to section 47.11 (3) of the regulations.

(a) produced from potato tissue culture material which has been tested and found free of bacterial ring rot, PSTVd and viruses;
(b) laboratory tested within 12 months prior to the completion of the multiplication process and found free of bacterial ring rot, PSTVd and viruses;
(c) produced in an aseptic or **protected environment**;
(d) visibly free from varietal mixtures;
(e) free from pathogenic bacteria or viruses, saprophytic contamination or other symptoms of diseases that could affect the quality of the material; and

(f) if produced in a **protected environment**,
  (I) inspected by an inspector at least once during the growing period; and
  (ii) grown in a medium that has not been previously used.

Nuclear stock equivalent: "cuttings or plants that were produced in a **protected environment** or from tubers or **selected clones**, and that were determined by laboratory tests to be free from any disease that could affect the quality of the seed."(refer to section 47.2 (a). of the regulations)

Official protocol: protocols that must be followed by laboratories that are accredited by the CFIA to perform diagnostic tests outlined in this memo.

Primary infection: The first infection of a plant by the overwintering or oversummering pathogens.

Protected environment: means a facility for which there are appropriate procedures and physical barriers to prevent the entry of plant pathogens and insects.

Secondary infection: Any infection caused by inoculum produced as a result of a **primary infection** or a subsequent infection.

Selected clones: **mother plants** selected in a certified field following quality and freedom from disease criteria: this selection process is usually known as **clonal selection**.

Tuber unit: means the separate pieces of one tuber that are planted consecutively in two or more hills in a row.

VI. LIST OF APPENDICES

Appendix 1: A flowchart for the production of Pre-Elite seed potatoes from other sources than Nuclear Stock (nuclear stock equivalent).

Appendix 2: Sampling procedure for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff 1914) Davis, Gillaspies, Vidaver & Harris 1984, the causal agent of bacterial ring rot, in nuclear stock equivalent seed potatoes.

Appendix 3: Sampling procedure for viruses and viroid (PSTVd) testing of nuclear stock equivalent seed potatoes.

Dr. J.E. Hollebone
Director
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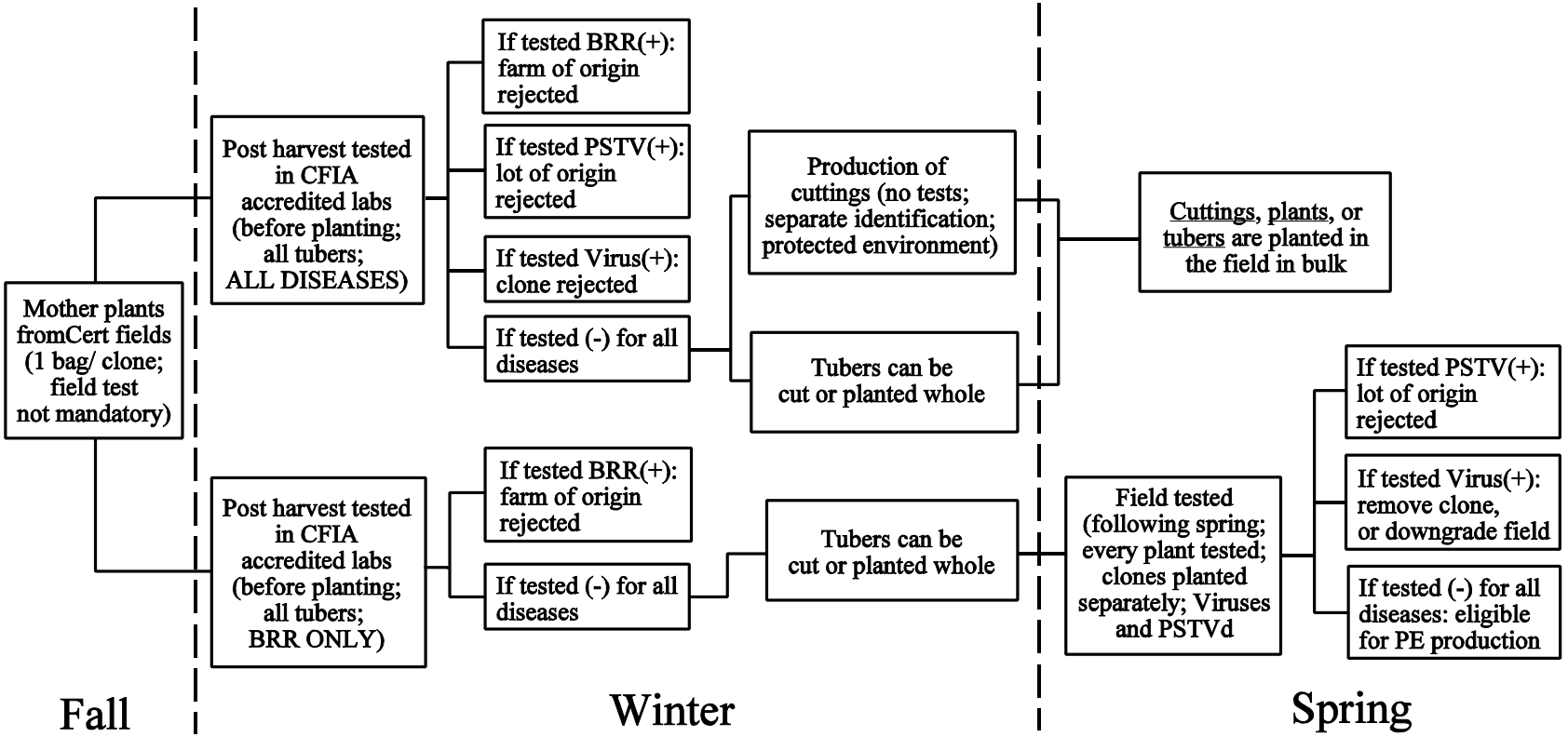
Attachment

APPENDIX 1

A flowchart for the production of Pre-Elite seed potatoes from other sources than Nuclear Stock (nuclear stock equivalent).



APPENDIX 2

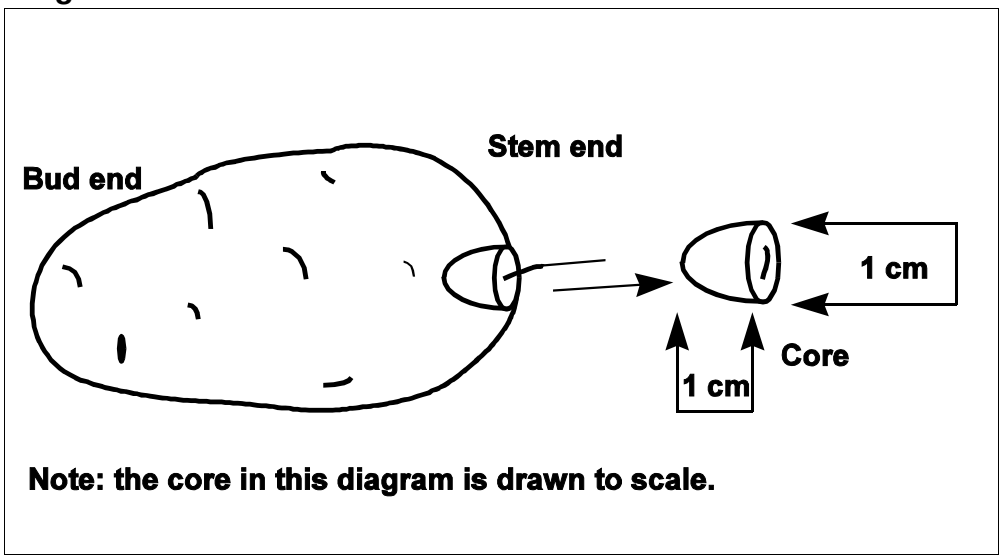
Sampling procedure for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff 1914) Davis, Gillaspies, Vidaver & Harris 1984, the causal agent of Bacterial Ring Rot (BRR), for nuclear stock equivalent seed potatoes.

Sampling

1. Only tubers will be tested for BRR. All the tubers from the **selected clones** must be tested; therefore it is presumed that coring will be done by the grower and the cores sent directly to the lab. When cores are sent to the lab, cored tubers should be retained by the grower for further investigation.

2. Cores must be taken at the stolon attachment site and must be cone or semi-spherical in shape, approximately 1 cm in diameter at the top and 1 cm deep (see diagram 1). Each core should weigh between 0.5 - 1.0 g, and include as much of the vascular ring radiating from the stolon attachment as possible.

Diagram 1: How to take a core from a tuber.



Combination of samples

1. Because follow-up measures on a positive sample (for BRR only) are taken on a lot of origin basis only, it is not necessary to bag and submit the samples to the lab by **clone**; **clones** from the same lot of origin can be submitted to the lab as one sample. However, **clones** should be identified on the bag.

APPENDIX 2 (cont'd)

2. Each sample is logged in separately by the lab; however, according to **official protocols,** samples may be combined by the lab for testing purposes.

3. The maximum number of cores that can be combined by the laboratory for testing is 200 (bags containing more than 200 cores will be subdivided). However, positive tests must be linked to a lot number for follow-up: backup material (tubers already cored and stored by the lab or by the grower) may be used to investigate positive composite samples. Samples from different farm units may not be combined into one laboratory sample.

4. Many options being available for sample submission to the lab (some that could help reduce the cost), it is advisable to contact the lab to make arrangements.

Packaging and shipping

1. To assure sample continuity from the field to the lab it is crucial to follow proper packaging, shipping, and identification procedures. If sample integrity is in question, the sample will be discarded, and must be resubmitted.

2. Because cores, dried and wrapped in paper towels, can be kept in cold storage (4ºC) for up to a maximum of 14 days before processing by the laboratory, keeping them refrigerated at all times is important. Samples can be kept for such an extended period only if no decay is observed. Cores and stems that become decayed during storage will be discarded by the laboratory and a new sample requested.

3. Make sure cores or stems are as dry as possible before packing. Cores should be wrapped in paper towels and shipped in plastic bags. As already stated, cores should be bagged by lot of origin. Each bag should be properly identified (see section on *Identification* below). Close and refrigerate the bags as soon as possible, but no more than two hours after coring. The bags should be kept in cold storage (4ºC) long enough (overnight) and in such a way (well spread) that the complete sample is brought down to 4ºC before packing. Make sure that the sample size indicated on the bags is correct: the lab will only accept a 2% deviation from this number.

4. Bags are then packed loosely in insulated cardboard boxes. Ice-packs should be included on top of the samples (cold air is moving downward), but should be sufficiently insulated from the samples so that freezing is avoided.

APPENDIX 2 (cont'd)

5. Do not forget that ice-packs are effective only for a short period: 24-48 hours depending on insulation. Shipment of samples must be postponed if the package is likely to be held in transit over a holiday or weekend, except if refrigerated storage is available.

6. When more than one sample is packed in the same container, a complete content list of samples submitted must be placed on the top of each shipping container or with the bill of lading; this list should be signed by the collector.

7. To ensure sample continuity, packages should be properly sealed so that nobody can open or alter them while in transit (transportation) without the laboratory being able to notice it upon arrival at the lab. If sample integrity is in question, the sample will be discarded and a new sample must be submitted.

8. When outside temperatures can go below the freeze point (0ºC or 32ºF), freezing must be avoided: the laboratory will reject any samples showing signs of freezing.

Identification

1. As already stated, samples from each lot of origin must be submitted in a closed, separate bag, and individually labelled. The following information is required on each label:
   - Grower's name (exactly as per the current application for Seed Potato Crop Inspection.)
   - Variety name
   - Certification number of the lot of origin and clones
   - Identification of the clones present in the bag
   - Class (to be assigned/already assigned)
   - Number of cores submitted
   - Date collected
   - Grower's or representative's signature
   - Test for which sample is submitted.

2. NOTE: samples not properly identified will not be processed by the lab until correct identification is received.

APPENDIX 3

Sampling procedure for viruses and viroid (PSTVd) testing of nuclear stock equivalent seed potatoes.

Sampling:

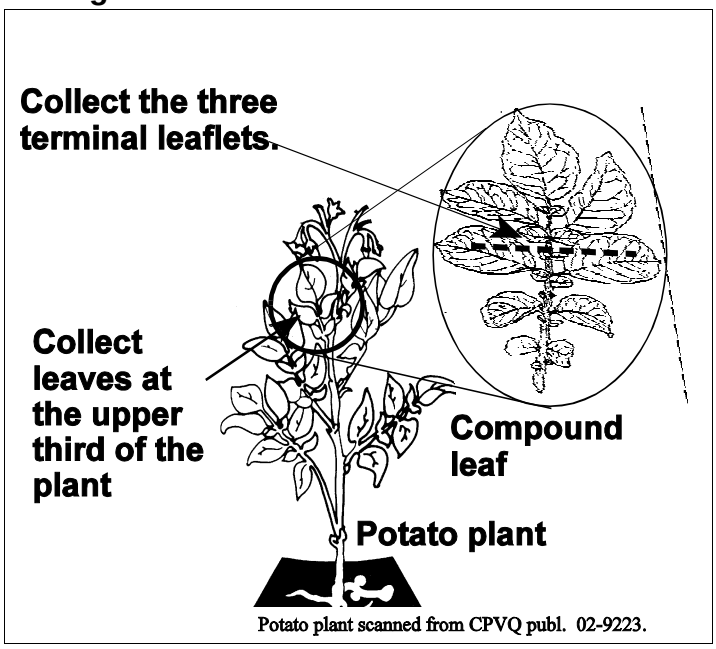
1. In all situations (field or greenhouse) where sampling has to be done for **nuclear stock equivalent** seed potatoes every **tuber unit** must be sampled.

2. Testing will be done on leaf or sprout material. The terminal three leaflets from a fully expanded young compound leaf are required from a plant from each **tuber unit** (See diagram 2). On arrival in the laboratory, acceptable samples must have at least 2 cm^2 of intact leaf tissue per leaflet. Slight wilting or breakdown at the leaf margins is acceptable.

   Leaves are collected from the upper third of the plant; this can be done as soon as the first compound leaves open, and always before plants begin senescing. When testing is done in the field sampling should be made early enough so that any removal pursuant to positive results can take place before cross contamination, by any means (insects, mechanical transmission by machinery, plants touching each other . . .) can happen.

   Sprouts of at least 1.5 cm have to collected from tubers that were maintain between 18-25 ^oC.

Diagram 2: How to sample leaves for virus testing.



3. Be advised that in most cases, according to official protocols, testing must be done within 72 hrs from leaves or sprouts collection: check with your local lab for details.

APPENDIX 3 (cont'd)

Packaging:

1. According to **official protocols** composite testing is possible. Options are available that can help reduce the cost: contact your local laboratory to make arrangements. However when a test is positive it must be linked to a clone. When composite samples of more than one **clone** are tested (to lower costs), backup material (only the terminal leaflet is used for the first test, leaving 2 leaflets for further analysis) may be used to investigate positive samples.

2. Leaves/sprouts must be bagged by **clone**, and the clone properly identified. Loosely folding over the opening of the bag and stapling shut is a good way of sealing the bag. The bag should not be sealed airtight, particularly if it is warm or damp; if necessary make breathing holes.

3. The leaf/sprout samples must be cooled (BUT NOT FROZEN) to 5^{o}C as soon as possible. It must be done within the hour of picking the leaves or the sprouts(particularly on warm days). If "ice-packs" are used, they should be insulated with two or three layers of paper or other packing material and be placed in the middle or top of the cooler. Two 6" x 6" "ice-packs" per cooler are usually sufficient. Avoid packing the leaves too tightly.

Shipping:

1. If the leaves are to be shipped by courier, the leaves should be held overnight in a refrigerated storage. For shipment, the bags should be packed loosely in the Styrofoam containers and place in cardboard boxes. "Ice-packs" should be included, but should be sufficiently insulated so as not to freeze the leaves.

2. Don't forget to respect the 72 hrs maximum period indicated above (*sampling*, 3.)

Identification: (to assure sample continuity from the field to the lab)

1. Every bag should contain the following information: grower or company name, the grower's signature, delivery date, variety, class and certification number. As indicated above, all **clones** must be identified separately.

2. A complete content list must be placed on the top of the samples or with the bill of lading, and signed by the collector.

3. Collection and submission dates of every sample must be provided.

4. Packages should be properly sealed.

APPENDIX 3 (cont'd)

***Notes**:*

1. SAMPLES NOT PROPERLY IDENTIFIED WILL NOT BE PROCESSED UNTIL CORRECT IDENTIFICATION IS RECEIVED.
2. IF SAMPLE INTEGRITY IS IN QUESTION, THE SAMPLE WILL BE DISCARDED, AND MUST BE RE-SUBMITTED.
3. SHIPMENT OF SAMPLES MUST BE POSTPONED IF IT IS APPARENT THAT THE PACKAGE WILL BE HELD IN TRANSIT OVER A HOLIDAY OR WEEKEND.