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	<b>(EFFECTIVE DATE)</b>  <b>July 15, 2005</b> <b>( 1<sup>st</sup> Revision)</b>
Title <b>Sampling requirements for PVY and PVY<sup>N</sup> testing in accordance with the “Canada / US Management Plan for Potato Viruses That Cause Tuber Necrosis”.</b>	

File

## **SUBJECT:**

This directive describes the requirements for collecting samples, from seed potato crops entered for certification, for the purposes of PVY and PVY<sup>N</sup> testing in accordance with the “Canada / United States (US) Management Plan for Potato Viruses That Cause Tuber Necrosis” dated October 25, 2004 (Management Plan). It includes guidelines for sample collection, identification, shipment and reporting of test results.

*This revision is made to provide direction on the change in sample collection intensity resulting from the Management Plan. Instead of sampling, as in previous years, 400 leaves from one Pre-Elite class planted crop from a group of 10 or less, the same 400 leaves sample must be prorated over all the crops of a group.*

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**Review**

This directive will be reviewed every three years unless otherwise needed. The next review date for this directive is July 15, 2008. The contact for this directive is Joanne Rousson. For further information or clarification, please contact the Potato Section.

**Endorsement****Approved by:**

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Director  
Plant Health Division

**Amendment Record**

Amendments to this directive will be dated and distributed as outlined in the distribution list below.

**Distribution**

1. Directive mail list (Regions, PHRA, USDA)
2. Provincial Government, Industry (via Regions)
3. National Industry Organizations (determined by Author)
4. Internet - CFIA website

**Introduction**

Canada's seed potato certification system ensures that high quality seed potatoes, free of a number of important diseases, are produced in accordance with the standards prescribed in the *Seeds Regulations Part II*, and in conformance with the provisions of the *Plant Protection Act* and *Plant Protection Regulations*. To this end, Canada has in place testing requirements for the tobacco vein necrosis strain of potato virus Y (PVY<sup>N</sup>). Canada has made a commitment, through a bilateral agreement with the US under the Management Plan, to collect and test for the presence of PVY and PVY<sup>N</sup> second generation seed potato crops. Second generation field crops in Canada are in the majority of cases those planted with Pre-Elite class seed potatoes.

In 1994 Canada and US plant protection officials adopted a PVY<sup>n</sup> management plan which was subsequently amended in 2001. Recently, as a result of bilateral discussions on a uniform approach to regulate viruses causing tuber necrosis, the "Canada/US PVY<sup>N</sup> Management Plan" (1994, revised in 2001) has been superseded by the "Canada/US Management Plan for Potato Viruses that Cause Tuber Necrosis" (Management Plan). One of the elements of the recently adopted Management Plan stipulates a revised PVY and PVY<sup>N</sup> sampling standard. All seed potato

certification agencies in the US and Canada are proceeding with the adoption of the PVY and PVY<sup>N</sup> testing requirements as specified in the Management Plan.

Continued participation in the Management Plan by the CFIA will result in continued benefits for the seed potato industry in Canada, including improved export opportunities and reliable sources of PVY<sup>N</sup>-free seed stocks, as per established sampling and testing standards.

This policy directive is intended to clarify and describe the PVY and PVY<sup>N</sup> sampling and testing requirements for farm units planting Pre-Elite class seed potatoes.

**Scope** This directive is intended for use by Canadian Food Inspection Agency (CFIA) staff and Canadian producers of seed potatoes.

**References** Department of the Secretary of State of Canada. *The Canadian Style: A Guide to Writing and Editing*. Toronto, 1993.

Canada / US - Management Plan for Potato Viruses that Cause Tuber Necrosis, October 25, 2004 version.

### Definitions, Abbreviations and Acronyms

CFIA Canadian Food Inspection Agency

Crop Breeder's Selection seed potatoes, or a variety and class of seed potatoes, growing in an aseptic environment, a protected environment or in one or more fields of a farm unit

Farm Unit (A) a single tract of land operated for the production and marketing of seed potatoes under the control of a grower, or  
(B) a number of separate tracts of land operated as a single unit, with the use of common equipment, facilities or storage, for the production and marketing of seed potatoes under the control of the same grower

Field The identifiable area of land on which Breeder's Selection seed potatoes, or seed potatoes of a particular variety and class, are planted or have been produced

Grower An individual, a cooperative, a corporation or a partnership that grows seed potatoes.

Lot The quantity of harvested seed potatoes of a variety and class that is identifiable by one certificate number or the quantity of Breeder's Selection seed potatoes that are identifiable by one certificate number

Management Plan "Canada/US - Management Plan for Potato Viruses that Cause Tuber

Necrosis”, October 25, 2004 version.

PVY <sup>N</sup>	The tobacco vein necrosis strain of potato virus Y (a type species of the Potyvirus genus of plant viruses, family Potyviridae)
Sample	Leaves or tubers collected for PVY and PVY <sup>N</sup> testing from a crop or lot. [For the purpose of this directive, it can be 1 - 400 (±10 %) leaves or tubers].
Sister Crop	Crops or fields of potatoes planted from the same seed source (i.e., the parent lot) whether grown on the same or a different farm unit

## 1.0 General Requirements

### 1.1 Legislative Authority

*Plant Protection Act, s.c. 1990, c.22*

*Plant Protection Regulations, SOR/95-212*

*Seeds Act ( R.S. 1985, c. S-8 )*

*Seeds Regulations (C.R.C., c. 1400), Sections 45-62*

*Canadian Food Inspection Agency Fees Notice, Canada Gazette, Part 1 (05/13/2000)*

### 1.2 Fees

The CFIA charges fees in accordance with the *Canadian Food Inspection Agency Fees Notice*. Anyone requiring other information regarding fees may contact any local CFIA office or contact the Internet website at [www.inspection.gc.ca](http://www.inspection.gc.ca).

There are no fees for the laboratory tests conducted on samples submitted in accordance with this policy directive and the Management Plan. However, pursuant to the *Canadian Food Inspection Agency Fees Notice*, part 12, section 12, a fee of \$5 per sample collected for the purpose of PVY<sup>N</sup> testing will be levied.

## 2.0 Policy

The objective of this policy is to provide direction to CFIA staff to achieve full compliance with section 3.1.3 of the Management Plan and provide the Centre of Expertise for Potato Diseases in Charlottetown with high quality leaf samples representative of the crop being examined.

The Management Plan replaces the previous Canada / USA PVY<sup>N</sup> Management Plan and includes a modified sampling regime which requires collecting material from all seed crops planted with Pre-Elite class seed potatoes.

In previous years, the *Seeds Regulations Part II* have stipulated that each crop planted

with Pre-Elite class seed potatoes would be subjected to laboratory testing for PVY<sup>N</sup>. However, the revised *Seeds Regulations Part II*, published in 2002, no longer contain this requirement. Therefore, the sampling approach to be adopted in light of this regulatory change will follow only the requirements described in the Management Plan. It is now the responsibility of the National Plant Protection Organizations of the participating countries to ensure that the requirements of the bilateral agreement are met. Therefore, these requirements are now mandated pursuant to the *Plant Protection Act and Regulations*. However, pursuant to the *Seeds Regulations Part II* section 52.(5) (d), seed certification will not be granted for lots confirmed to be infected with PVY<sup>N</sup>.

The Management Plan states that all crops planted with Pre-Elite class seed, Field Generation 2 (FG2), on each seed farm unit must be tested for the presence of PVY and PVY<sup>N</sup>. In addition, it stipulates that the total number of leaves from all samples from the group of 10 crops or less from a farm unit is 400 leaves. Therefore, in practice this means that for every set of 1-10 crops planted, a total of 400 leaves must be taken (i.e., even if only 11 crops on a farm are planted with Pre-Elite seed lots, then 800 leaves must be collected).

## 2.1 Sample Collection

2.1.1 Sample collection in accordance with this policy directive and the Management Plan is the responsibility of the CFIA. Therefore, all samples collected for the purpose of PVY and PVY<sup>N</sup> testing in accordance with this directive must be collected by, or under the supervision of, a CFIA inspector. However, the grower is encouraged to assist CFIA inspectors in the collection of samples.

2.1.2 The Management Plan stipulates that all FG2 crops, in Canada it translates to all crops entered for certification and planted with Pre-Elite class seed potatoes, on each farm unit will be tested for PVY and PVY<sup>N</sup>. Therefore, a prorated sampling regime equating to 400 leaf collected randomly over the area (# Ha) covered by every set of 1-10 crops planted with Pre-Elite class seed potatoes will be adopted. Where the crop(s) consists of less than 400 plants, at least one leaf per plant must be collected. Appendix 1 provides details on prorating the sample collection over the number of crops required to be sampled per farm unit.

2.1.3 For each group of 1-10 crops identified by the inspector, a sample of 400 leaf triplets or one leaf triplet per plant, if there is less than 400 plants, (section 2.1.5) is required to be collected and tested. For practical purposes, collection of samples during the first or final inspection, or at any time between these inspections, will be sufficient to meet the terms of the Management Plan. Note carefully the recommended weekly shipping days (section 2.3.3) as this will dictate when samples should be collected.

2.1.4 Leaf surfaces must be completely free of water droplets prior to collecting samples.

- 2.1.5 Each individual leaf collected is to consist of the terminal three leaflets from a **fully developed** compound leaf on the upper part of the plant. Leaves that are not fully developed are not suitable for testing and must not be collected. These leaflets should remain attached to the petiole of the compound leaf upon which they were borne.

**Note:** The required average leaflets's length should be no less than four centimetres. In cases where leaflets are less than four centimetres, the number of leaflets collected should be increased to compensate by providing a total tissue mass equivalent to three leaflets of four centimetres each (all leaflets must be attached to the same petiole). If there are difficulties in estimating the required mass, the entire compound leaf should be collected.

- 2.1.6 It is important that each leaf be collected in a manner that ensures samples are randomly distributed across the field, and that the sample will be statistically representative of the entire field. A statistically reliable sampling method is described in Appendix 2.
- 2.1.7 A variation of  $\pm 10\%$  from the identified sample size is considered acceptable as meeting the sampling and testing objectives. However, due to the possibilities of leaf tissue break-down during shipment, and the possibility of errors being made during counting, it is highly recommended to include an extra 10% to the number of leaves required (e.g. aim to submit 440 leaves for a sample requiring 400 leaves to allow for errors or rejections by the laboratory). If a sample consists of fewer than 360 leaves when it should have been 400 leaves, the sample may not be considered eligible for testing or as meeting the requirements and may be rejected from testing.

**Note:** counting errors can be largely avoided/minimised by the use of a tally counter.

- 2.1.8 During collection, samples should be accumulated by stacking carefully and evenly in plastic or paper bags. Each bag must be labelled inside and out in accordance with the requirements of section 2.2. If using plastic sealable bags, ventilation holes must be present in the surface. Some manufacturers of plastic bags for retail sale (for the purpose of refrigerating vegetables) are now offering finely perforated sealable plastic bags, which can be used very successfully for potato leaf samples. The larger leaf sample sizes, (i.e. 400 leaf triplets) can be divided equally between four or more bags. Each leaf triplet must be stacked evenly within the bag to minimize damage to the tissue as damage will accelerate the rate of tissue breakdown. A piece of paper towel may be included in each plastic bag to absorb any moisture that may develop.
- 2.1.9 Once the collection of a leaf sample is done, it should be placed in a refrigerator or cooler, with the bag's neck folded over loosely in an attempt to allow some air circulation while cooling. **Note:** leaf samples must **not** be frozen.
- 2.1.10 Once all required samples for a farm unit have been collected, appropriately labelled, and

cooled, the sample bags can be sealed securely and shipped or delivered by the CFIA inspector as per the requirements described in section 2.3.

- 2.1.11 If summer leaf testing is not carried out, or if the sampling method employed does not meet the above requirements or samples were not accepted for testing, a random selection of the required number of tubers (i.e., 400 + 10 % to allow for tuber decay, non-germination, etc.) must be taken at the time of harvest, following the sampling pattern described in Appendix 2. Such collection must be carried out by, or under the supervision of, an inspector. A minimum number of leaves (obtained from greenhouse-grown progenies) or sprouts from the tubers sampled will then be tested in accordance with the protocol specified in the Management Plan. Given the delays that would occur in generating the necessary results under such a scenario, growers would be well-advised to make every effort and all necessary arrangements to ensure that summer sampling is carried out in accordance with the above requirements.
- 2.2 Sample Identification and Labelling
- 2.2.1 Accurate sample identification and labelling is the responsibility of the inspector.
- 2.2.2 It is essential that each sample submitted can be traced back reliably to its current year farm unit and seed source. For this reason, great care must be taken to ensure the integrity of the sample collected, the identification labelling applied, and the method of marking samples (i.e., the label must be clear and legible on arrival at the laboratory).
- 2.2.3 Each sample bag must have an identification tag placed inside **and** a label fixed to the outside of the bag (or permanent marker used on a bag's integral label fields if present).
- 2.2.4 Each identification label (on both inside and outside of each bag) must bear the following information:
- the legend, "Sample for PVY and PVY<sup>N</sup> testing"
  - the grower's name and address
  - the CFIA-assigned grower number of the farm unit (that the seed has been planted on in the current season)
  - the variety name
  - the current year's lot number (that will become the CFIA-assigned certification number of the planted crop). This can be determined by reference to field number used on the current year's Application for Seed Potato Crop Inspection Growers Declaration (CFIA 1317)
  - the date the sample was collected
  - the grower's signature (Not absolutely necessary, nice to have if the grower is collaborating with the sample collection.)
  - the number of leaves to test.



- the name of the CFIA inspector who collected the sample or supervised its collection
- The CFIA inspector's signature.

### 2.3 Shipment of Samples (submission to laboratory)

2.3.1 Brief pre-shipment storage (if necessary) at a CFIA facility and shipping should be arranged by a CFIA inspector. Costs related to the shipment of the samples to the laboratory will be met by the CFIA at point of shipping. Samples must not be sent to the laboratory on a cash on delivery (COD) basis.

2.3.2 Efforts must be taken to minimise the potential break down of the samples during transit. Therefore, all samples sent to the laboratory must be shipped in coolers containing ice packs. Ice packs should be wrapped in paper towels or newspaper to avoid frost damage to any tissue coming into direct contact with the ice pack. **Note:** Ice packs should not be placed on top of samples, but should line the walls of the cooler.

2.3.3 Potato leaves can be stored in a standard refrigerator at 4 - 7° C for up to five days and still be suitable for testing. However, potato leaves stored in cooler box with an ice pack and paper towels may begin to break down after three days. Since the laboratory system requires 12-24 hours to process the samples, providing that they arrive early in the week, every effort must be made to reduce the period that leaf samples are stored in the portable cooler during shipping. The following recommendations should minimise the in-transit period:

- leaf samples should be collected no more than two days prior to shipping
- prior to shipping, the leaves should be stored in a refrigerator at 4 - 7° C and transferred to the portable cooler only immediately prior to dispatch
- the shipping service used must offer delivery times of 48 hour or less
- shipment of samples from point of origin should commence only on a Monday or Tuesday, in order to avoid arrival at the laboratory during the weekend

2.3.4 All samples should be shipped directly to the Centre of Expertise for Potato Diseases at the following address (the telephone and fax numbers should be made available to the courier):

Attention: PVY and PVY<sup>N</sup> Testing Coordinator  
Centre of Expertise for Potato Diseases  
93 Mount Edward Road  
Charlottetown, PEI  
C1A 7M8

Telephone: (902) 368-0950 / Facsimile: (902) 368-0295

2.3.5 On arrival at the laboratory, samples must be placed in a refrigerator at 4°C if the samples

cannot be tested immediately.

- 2.3.6 The laboratory at the Centre of Expertise for Potato Diseases, will, where day of arrival permits, proceed within 24 hours to test the samples in accordance with the protocol described in the Management Plan.
- 2.3.7 Should the condition of any samples be unsuitable for the appropriate testing to be performed, the laboratory will immediately communicate this to the Regional Inspector / Program Officer responsible for coordination of PVY and PVY<sup>N</sup> sampling. The regional coordinator must then contact the appropriate District Office in order that the inspector can proceed with collection of new samples. Time is of the essence if samples need to be re-collected, because the ability to detect the presence of PVY and PVY<sup>N</sup> using serological laboratory methods decreases as host plant material approaches senescence. If re-sampling is necessary but the appropriate window for sampling (2.1.3) has expired, post-harvest sampling and testing will be required, as described in section 2.1.11.
- 2.4 Reporting of Laboratory Test Results
- 2.4.1 Once the staff at the Centre of Expertise for Potato Diseases have completed all required testing on all samples received for PVY and PVY<sup>N</sup> testing, region-specific test results will be distributed to each respective regional coordinator. In addition, the laboratory will distribute a full report of the national testing results to the National Manager of the Potato Section, and to each Area Network Seed Potato Specialist. This reporting should take place by the end of September of each year. Individual growers should contact their inspector if they require confirmation of the testing results for their crop(s).
- 2.4.2 During the testing carried out by the Centre of Expertise for Potato Diseases, should any sample tested provide positive results for the presence of PVY<sup>N</sup>, this will be immediately communicated to the National Manager of the Potato Section, the Area Network Seed Potato Specialist, the Regional Program Officer and the regional coordinator, along with appropriate identification of the sample(s). Confirmatory tests will be initiated immediately on the suspect crop (section 2.4.3).
- 2.4.3 Should any sample submitted for testing under this directive and determined to be positive for the presence of PVY<sup>N</sup> during primary testing, the appropriate Area Network Specialist will contact the regional coordinator who will liaise with the District Office and the Centre of Expertise for Potato Diseases to ensure that the requirements of the Management Plan are implemented (**note**: these requirements may result in sampling of all the sister crop(s) and all Pre-Elite planted crops on the farm unit at 400 leaves). These requirements include the following:

- confirmatory testing on the crop, which involves further serological tests as well as molecular analyses and bioassays. **Note:** while awaiting the results of the confirmatory tests, the crop in question and any sister crop(s) are not eligible for certification.
- if confirmatory tests are negative, the crop and any sister crop(s) are declared PVY<sup>N</sup> negative and certification can proceed.
- if confirmatory tests are positive, the respective crop will not be eligible for certification. In addition, it must be confirmed that all sister crops have been tested in accordance with the Management Plan. Furthermore, all Pre-Elite planted potato crops on the affected farm unit must be sampled and tested at a rate of 400 leaves or tubers per crop as described in section 2.1.

### 3.0 Appendices

- Appendix 1 - Guidelines on sampling for PVY and PVY<sup>N</sup> testing based on number of crops planted with Pre-Elite class seed potatoes on a farm unit and entered for certification.
- Appendix 2 - Sampling method (grid pattern) recommended for PVY and PVY<sup>N</sup> sample collection

## Appendix 1

### Guidelines on sampling for PVY and PVY<sup>N</sup> testing based on the number of crops planted with Pre-Elite class seed potatoes on a farm unit and entered for certification.

In determining how many leaves should be collected per sample for PVY and PVY<sup>N</sup> testing purposes, table 1 outlines the number of samples to expect and the total number of leaves expected per group of samples based on the number of crops planted with Pre-Elite class seed on a farm unit.

**Table 1:** Number of seed crops planted with Pre-Elite class seed per farm unit requiring PVY and PVY<sup>N</sup> testing

Number of crops planted with Pre-Elite class seed potatoes on a farm unit. (# samples)	Total number of leaves/tubers* to collect.
1 to 10	400
11 to 20	800
21 to 30	1200
31 to 40	1600
41 to 50	2000
Additional . . .	Add 400 leaves for each additional composite of 1-10 crops.

\*Tuber samples are only to be collected if leaf sampling has not been carried out or was unsuccessful (section 2.1.11)

The number of leaves to collect per sample is based on the number of hectares planted per crop and the number of hectares per group of 1-10 crops. The examples below can be used to better understand how to determine the sampling rate and arrive at the 400 ±10 % leaves required for every group of 1-10 crops.

Example # 1:

Farm unit with **3 crops** entered for certification and planted with Pre-Elite class seed potatoes of the following field sizes: **Field 1: 0.7 Ha, Field 2: 1.2 Ha, Field 3: 0.3 Ha.**

Crop I.D.	# Hectares	# leaves required <sup>1</sup>	Sampling rate	# leaves/sample (crop)
<b>1</b>	0.7	440 leaves for this group of 10 crops or less.	200 leaves/Ha. (440 ÷ 2.2= 200)	140 <sup>2</sup>
<b>2</b>	1.2			240 <sup>2</sup>
<b>3</b>	0.3			60 <sup>2</sup>
<b>Total</b>	2.2			440 <sup>3</sup>

1- Includes the 10 % extra for leaf count errors and possible deterioration in transit.

2- Sampling rate multiplied by the number of hectares of the specified crop.

3- Total number of leaves to collect for the three samples.

## Example # 2:

Farm unit with **12 crops** entered for certification and planted with Pre-Elite class seed potatoes of the following sizes: **Field 1: 0.7 Ha, Field 2: 2.2 Ha, Field 3: 0.3 Ha, Field 4: 0.3 Ha, Field 5: 0.8 Ha, Field 6: 0.7 Ha, Field 7: 0.7 Ha, Field 8: 0.4 Ha, Field 9: 0.1 Ha, Field 10: 0.2 Ha, Field 11: 1.1 Ha and Field 12: 0.1 Ha.**

Crop I.D.	# Hectares	# leaves required <sup>1</sup>	Sampling rate	# leaves/sample (crop)
<b>1</b>	0.7	440 leaves for this group of 10 crops or less.	69 leaves/Ha. (440 ÷ 6.4= 69)	48 <sup>2</sup>
<b>2</b>	2.2			152 <sup>2</sup>
<b>3</b>	0.3			21 <sup>2</sup>
<b>4</b>	0.3			21 <sup>2</sup>
<b>5</b>	0.8			55 <sup>2</sup>
<b>6</b>	0.7			48 <sup>2</sup>
<b>7</b>	0.7			48 <sup>2</sup>
<b>8</b>	0.4			28 <sup>2</sup>
<b>9</b>	0.1			7 <sup>2</sup>
<b>10</b>	0.2			14 <sup>2</sup>
<b>Total (first 10 crops)</b>	6.4			442 <sup>3</sup>
<b>11</b>	1.1	440 leaves for this group of 10 crops or less.	367 leaves/Ha. (440 ÷ 1.2 = 367)	404
<b>12</b>	0.1			36
<b>Total (11 - 20 crops)</b>				

1- Includes the 10 % extra for leaf count errors and possible deterioration in transit.

2- Sampling rate multiplied by the number of hectares of the specified crop.

3- Total number of leaves to collect for the three samples.

## Appendix 2

### Sampling method (grid pattern) recommended for collection of samples for PVY and PVY<sup>N</sup> testing

The *Management Plan* requires PVY and PVY<sup>N</sup> testing to be carried out on 400 plants per group of 10 crops or less. To account for the possibility of errors in collection, or tissue breakdown during transit, it is recommended that 440 leaves (this represents the number of plants required plus 10 % to allow for error/breakdown) be gathered from the identified crops (1-10). There is also a requirement to adopt a randomized, systematic grid sampling approach. This can be achieved by randomly selecting the starting point after which sample collection follows a systematic grid pattern to ensure that samples are collected from all sectors of the field.

The sampling rate (number of plants per hectare) should first be determined. This is calculated simply by dividing the required sample size of 440 leaves by the total area (in hectares) covered by the identified crops (1 -10), and rounding to the nearest whole number. For example, to collect a 440 leaves from three crops which are in total covering 1.4 hectares in size, the appropriate sampling rate per hectare would be 220 plants per hectare as shown below (see example 1, appendix 1).

$$\begin{array}{l} \text{Required sampling rate per hectare} \\ \text{(example) =} \end{array} \quad \frac{440}{2.2} = 200$$

Table 2 provides appropriate sampling rates for a range of approximate field(s) sizes. These sampling rates can be used to establish the sampling pattern described below, or the above calculation can be used to determine the exact sampling rate. Once the sampling rate is known, reference to table 2 allows appropriate grid dimensions for systematic sampling to be selected. For statistical reasons, the grid pattern used should result in the headland distance being about four times greater than the within-row distance. Table 2 shows different 4:1 grid dimensions to be adopted based on different sampling rates. If the exact field(s) size and sampling rate is not shown in the table, the closest size can be selected and the appropriate grid size used. Using these grid dimensions, a random starting position is used within each row, followed by collection of samples using the specified dimensions.

If, for example, it is determined that a field (e.g., 4 hectares) should be sampled at a rate of 100 plants per hectare then, as per table 2, individual leaf samples should be collected from plants located every 20 x 5 metres across the field, to provide the required 100 samples per hectare and to cover the entire field area. Although this meets the requirement to be systematic, it does not fulfil the requirement for random sampling. The random element is incorporated by selecting a starting point by replacing the numbers used in the grid pattern (in this case 20 x 5 metres) with random numbers **within the same range as the grid dimensions**. In this example, random numbers would be selected from the range 1 - 20 and 1 - 5, and then factored into the grid sampling equation in place of the standard 20 x 5 metre pattern. An example of selecting the starting point using a systematic, random approach based on this 100 plant per hectare sampling rate is provided following table 2.

**Table 2:** Examples of approximate sampling rates per hectare, idealised grid sizes and ranges for randomised starting points to serve as guidelines for collecting samples for PVY<sup>N</sup> testing.

Approximate field(s) size (hectares)	Sampling rate (plants per hectare)	Idealised grid size for sampling: headland x within-row distance (metres)	Range for random grid size starting points (metres)
0.1	4000	3.2 x 0.8	1-3 x 0.2-1
0.25	1600	5 x 1.25	1-5 x 0.25-1.25
0.50	800	7 x 1.8	1-7 x 0.5-2
0.75	534	8.7 x 2.2	1-9 x 0.5-2
1	400	10 x 2.5	1-10 x 0.5-2.5
1.25	320	11.2 x 2.8	1-11 x 0.7-3
1.5	267	12.3 x 3.1	1-12 x 0.7-3
1.75	229	13.2 x 3.3	1-13 x 0.7-3
2	200	14.1 x 3.5	1-14 x 1-4
2.5	160	15.8 x 4	1-16 x 1-4
3	134	17.3 x 4.3	1-17 x 1-4
3.5	115	18.7 x 4.7	1-19 x 1-5
4	100	20 x 5	1-20 x 1-5
4.5	89	21.2 x 5.3	1-21 x 1-5
5	80	22.4 x 5.6	1-22 x 1-6
6	67	24.5 x 6.1	1-25 x 1-6
7	58	26.5 x 6.6	1-27 x 1-7
8	50	28.3 x 7	1-28 x 1-7
9	45	30 x 7.5	1-30 x 1-8
10	40	31.6 x 7.9	1-32 x 1-8
11	37	33.2 x 8.3	1-33 x 1-8
12	34	34.6 x 8.7	1-35 x 1-9
13	31	36 x 9	1-36 x 1-9
14	29	37.4 x 9.4	1-37 x 1-9
15	27	38.7 x 9.7	1-39 x 1-10
16	25	40 x 10	1-40 x 1-10
17.5	23	41.8 x 10.5	1-42 x 1-11
20	20	44.7 x 11.2	1-45 x 1-11
22.5	18	47.4 x 11.9	1-47 x 1-12
25	16	50 x 12.5	1-50 x 1-13
30	14	54.8 x 13.7	1-55 x 1-14
40	10	63.3 x 15.8	1-6 x 1-16
50	8	70.7 x 17.7	1-71 x 1-18



### Example of collecting samples in a systematic, random pattern

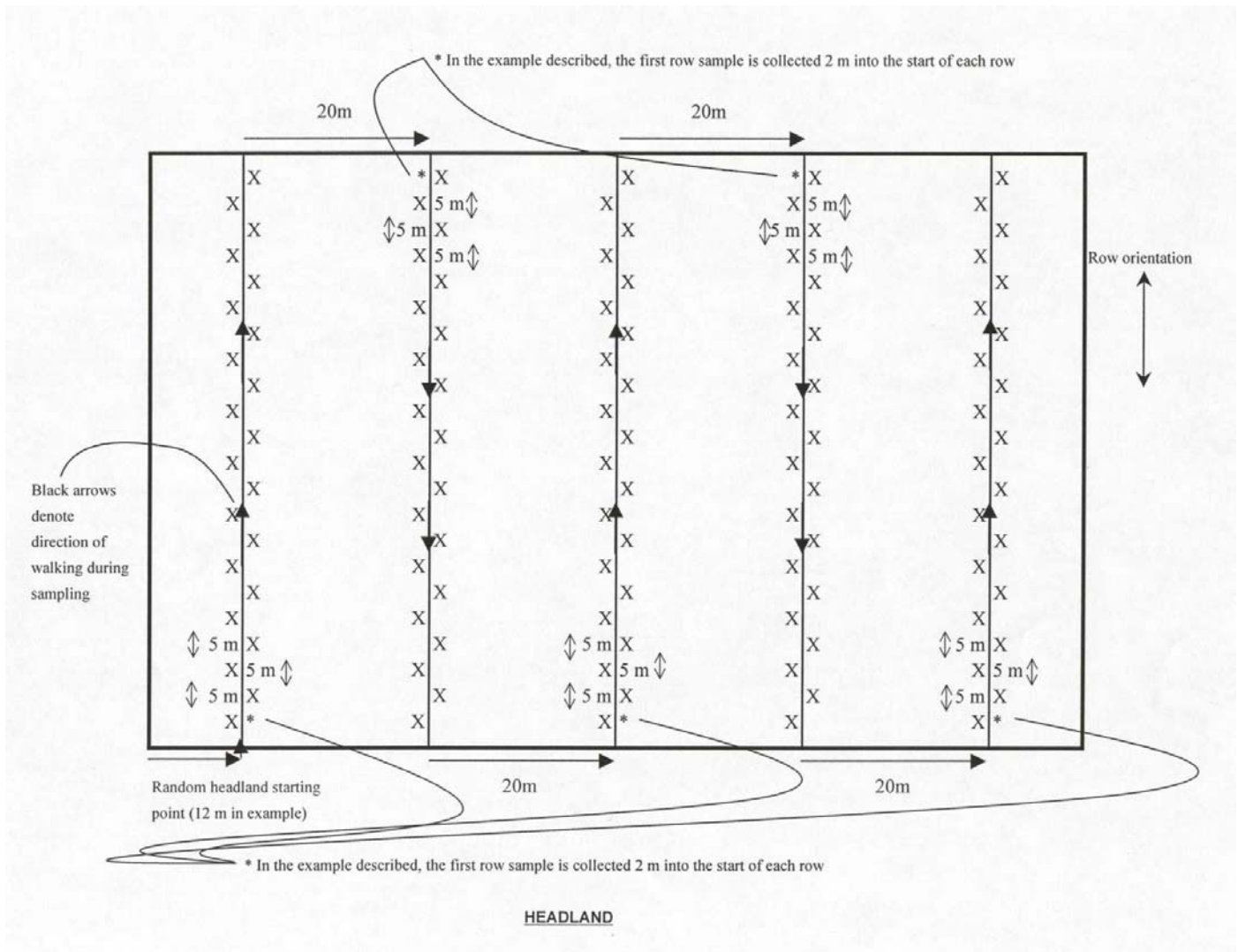
This example relates to a field size of 4 hectares, resulting in a sampling rate of 100 plants per hectare, with a prescribed grid size of 20 x 5 metres (table 2).

**Note:** the following example is also represented schematically in figure 1.

The sample collector would stand at one corner of the field, beside the first plant at the end of the first row. The collector would then select a random number between 1 and 20 (e.g., 12) and walk this distance (12 metres in this example) across the headland (i.e., across the ends of rows). The collector would then turn to face into the field, looking between the two nearest rows of potatoes. A random number between 1 and 5 (e.g., 2) would then be selected. The collector would walk this distance (2 metres in this example) in from the headland between the rows. This point would represent the randomly selected starting point of the 20 by 5 metre grid pattern. The random number for row distance (2 m) would then be retained and used to determine the starting point within each row. However, beyond these two random elements, the standard grid size (in this case 20 x 5 m) is used to collect samples from the field.

At this starting point (12 x 2 m from the corner in this example), a terminal leaf triplet would be collected from the nearest plant from the row to the collector's left. Following this, the standard 5 metre row distance would be adopted for samples subsequently collected within the row. Therefore, the collector would proceed a further 5 metres into the field between the same two rows, and collect a sample from the nearest plant from the row on the right. The collector would then continue along between the rows in this manner, to the end of the field, collecting a sample at every 5 metres, alternating from the row on the left to the row on the right for each sample selected. When the end of the field is reached, the collector would walk a further 20 metres across that headland and then walk down the closest row by the original randomly selected number of metres for row starting point (in this example, 2 metres). The first sample for that row would then be collected, followed by further samples at 5 metre intervals (again, alternating between the rows on the left and right). In this way, the sample collector would cover the entire the field at 20 m intervals across the headland, collecting a sample every 5 m along the rows.

In this example, regardless of the shape of the field, a 20 by 5 metre grid pattern will lead to a sampling rate of 100 plants per hectare. The statistical requirement for a grid pattern resulting in the headland distance being about four times greater than the within-row distance is also retained.



**Figure 1:** Schematic representation of suggested PVY and PVY<sup>N</sup> sampling method reflecting example described in the text and summarised below:

**Example (summary)**

Sampling rate (table 2):

Field size of 4 hectares requires sampling rate of 100 plants per hectare resulting in a sampling grid pattern of 20 x 5 metres

Randomised starting point (table 2):

Headland range between 1 and 20 metres (12 m selected)

In-row range between 1 and 5 metres (2 m selected)

Obviously, paced measurements will never be exact and, therefore, once the samples for each field have been collected using an estimated grid size, it is likely that either too few or too many leaves will have been collected. Therefore, the total number of samples collected for the field should be carefully kept track of during leaf collection (a tally counter is strongly recommended for this purpose). Samples may be randomly skipped during collection, or “top-up” samples randomly collected once the grid pattern has been completed, if this is necessary to ensure the total sample consists of 440 leaves collected for each field.

If summer leaf sampling was not carried out (section 2.1.11) it is necessary to randomly select 400 tubers from the field at harvest. The above sample pattern should still be adopted (just as if leaves were being collected). However, extra tubers (10 % suggested) should also be collected to ensure that a minimum of 400 grow out samples (one from each tuber) will be available for testing (i.e., to accommodate for some lack of germination, etc).

The objective of sampling is to provide the laboratory with high quality leaf samples, that are truly representative of the crop being examined. During summer leaf sampling, it is the responsibility of the grower and the inspector collecting or supervising the collection of samples to ensure that this objective is achieved.