Procedures for standard evaluation trials of advanced potato clones

An International Cooperators' Guide

International Potato Center (CIP)

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International Potato Center
Apartado 1558, Lima 12, Peru
cip@cgiar.org
www.cipotato.org
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Credits:
Editors: Merideth Bonierbale, Stef de Haan, Anne Forbes
Copy editing / proof reading: Linda Crissman
Design and layout: Thomas Zschocke, Alfredo Puccini
Photographs: International Potato Center (CIP)

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Making suggestions

Suggestions for improving the procedures for standard evaluation trials described in this publication are welcome. If national procedures for variety registration require the evaluation of additional performance parameters, the protocols and evaluation scales for collecting those data may be added to this guide.

Please send them to the Germplasm Enhancement and Crop Improvement Division at the address listed below:

International Potato Center
Apartado 1558, Lima 12, Peru
cip@cgiar.org
www.cipotato.org

CIP database

A potato-breeding and selection network on information exchange (CIPPEX) about advanced clones will be hosted at the headquarters of the International Potato Center (CIP). CIP will establish a geo-referenced database to support the storage and exchange of resulting information.

Please send your data to the International Potato Center at one of the contact addresses listed below.

CIP contact addresses

International Potato Center (CIP)

CIP Headquarters

Apartado Postal 1558
Lima 12, Peru
cip@cgiar.org

Latin America & the Caribbean (LAC)

Regional Office Peru
Apartado 1558
Lima 12, Peru
cip@cgiar.org

Liaison Office Ecuador
Apartado 17-21-1977
Quito, Ecuador
cip-quito@cgiar.org

Sub-Saharan Africa (SSA)

Regional Office Kenya
c/o ILRI
P.O. Box 25171
Nairobi 00603, Kenya
cip-nbo@cgiar.org

Liaison Office Uganda
c/o PRAPACE
P.O. Box 22274
Kampala, Uganda
prapace@prapace.co.ug

South, West and Central Asia (SWCA)

Regional Office India
NASC Complex
DPS Marg, Pusa Campus
New Delhi 110012, India
cip-delhi@cgiar.org

Liaison Office Uzbekistan
c/o ICARDA-CAC
P.O.Box 4564
Tashkent 700000, Uzbekistan
c.carli@cgiar.org

East, and Southeast Asia & The Pacific (ESEAP)

Regional Office Indonesia
Kebun Percobaan Muara
Jalan Raya Ciapus
Bogor, Jawa Barat, 16610 Indonesia
cip-eseap@cgiar.org

Liaison Office China
c/o The Chinese Academy of Agricultural Sciences
Zhong Guan Cun South Street 12
West Suburbs of Beijing
Beijing, People's Republic of China
cip-china@cgiar.org

Liaison Office Vietnam
Nha so 10, ngo 283
Doi Can, Ba Dinh
Hanoi, Vietnam
tnguyen@cgiar.org

Liaison Office Philippines
CIP-UPWARD
c/o IRRI DAPO 7777
Metro Manila, Philippines
cip-manila@cgiar.org

Introduction International Cooperators Guide (ICG)

About the ICG

The International Cooperators' Guide provides protocols for cooperating scientists to collect and exchange data on the performance of advanced potato germplasm. It was designed to facilitate standard and uniform procedures for collecting and sharing a minimum set of basic data.

Objectives of the ICG

- To stimulate and facilitate the use of standardized procedures for advanced potato germplasm evaluation.
- To promote uniform data sharing between institutions dedicated to advanced potato germplasm development and evaluation.

Users of the ICG

This guide has been developed for diverse users, ranging from non-specialists to specialists, involved in the evaluation of advanced potato germplasm for future regional or national variety release. Potential users include crop scientists, agronomists or students committed to the evaluation of locally-developed or CIP-provided advanced clones, local varieties or potato germplasm from other sources.

Contents of the ICG

This guide contains guidelines and protocols concerning the planning of evaluation trials (Chapter 1), tuber yield assessment (Chapter 2), post-harvest quality and storability evaluation (Chapter 3), late blight resistance assessment (Chapter 4), bacterial wilt resistance assessment (Chapter 5), leaf miner fly resistance assessment (Chapter 6), and virus resistance assessment (Chapter 7). Furthermore, the annexes in this guide provide additional detailed information for consultation and standard datasheet formats for data collection.

Planning Evaluation Trials

Introduction

Careful planning is an essential first step prior to the actual installation of evaluation trials. A representative location and season, sufficient healthy planting material, and a suitable trial design are necessary. Soil preparation and sowing practices must be appropriate, with provision made for sufficient labor. Initial data, such as the type of clones used and a map of the area, should be recorded.

Planning is also required for maintenance of the trial and collection of data during the crop growth cycle. From installation of the trial until harvest, there must be sufficient labor for weeding, hilling, pest and disease management and irrigation carried out according to local, commercial or recommended practice. The process of data collection and registration should be planned.

Plans for use and management of post-harvest data should be in place, with consideration given to data analysis, interpretation, sharing and feedback.

Conducting evaluation trials

Location(s)

Location(s) should be determined by the objective(s) of a trial. Yield trials should be installed in regions that are representative of commercial potato production, while intentional exposure trials should be installed in areas where disease or pest pressure is high. On the other hand, genotype by environment (GxE) trials require multiple locations.

Materials

Many of the necessary materials can easily be anticipated, but some are largely dependent on the type of trial. The following is a general checklist:

- Seed of potato clones and control varieties
- Field book, map and pen
- Fertilizers, pesticides or other inputs

- Equipment for climate measurement
- Net or paper bags for sampling and/or harvest
- Scale and calculator

Producing seed

Because the technology used for seed production should be adapted to the local environment, there are many methods to produce healthy seed. In highland environments, high quality seed tubers for evaluation trials can be obtained from healthy plants identified in the field during a previous growing season (positive selection). Seed can also be produced in net houses by using high-tech systems such as hydroponics, aeroponics and by multiplying plants derived from health-tested in vitro cultures or pre-basic tubers.

If no local experience with potato seed production exists, contact with a national specialist and referral to seed production manuals is recommended (Loebenstein *et al.*, 2001).

Multiplication plot locations

When healthy seed is needed, it can be produced on site in a seed multiplication plot. Seed production should be anticipated for all the trials for a three-year period and a seed production scheme developed that will meet this demand.

The seed production plot should be located in an area free of virus vectors, with minimal disease/pest pressure, and preferably isolated from potato production fields. The seed plot should not have been planted with solanaceous crops in the previous two years. The seed plot should not be used for evaluation during the growing season, as frequent entry increases the risk of spreading diseases.

Multiplication plot materials

In the season before evaluation trials, healthy source plants of each test clone should be selected from in-vitro cultures, fields or net houses. A conservative multiplication rate of 5 seed size tubers/plant is used to plan the number of source plants to be used in each multiplication cycle.

The minimum seed requirements for different types of evaluation trials are given in Table 1.

Table 1. Minimum healthy seed per clone for evaluation trials.

Evaluation Trials	Year		
	1	2	3
Tuber yield	40	240-400	>400
Late blight	20	40	
Bacterial wilt	20	100	
Leaf miner fly	20	90	
PVX/PVY virus (field)	20	-	-
PLRV virus (field)	20	-	-
PVX/PVY virus (greenhouse)	7		

Note that healthy seed is not required for seasons 2 and 3 of the virus resistance trial. At the end of each season, the seed is kept and is used for the next planting. The purpose is to obtain after 2 seasons at least 100 seeds with high virus infection rates that will allow the implementation of the virus intentional trial during the 3rd season.

Multiplication plot field management
Standard agronomic practices and strict procedures to protect the seed plot from pests, diseases and vectors should be applied. Off-types and plants with virus infection are eliminated through roguing at about 45 to 55 days after planting before the canopy closes. Health status is confirmed using a serology index (ELISA or other).

After harvest, the seed tubers are labeled and stored in a well-protected storage environment separated from consumption potatoes.

Experimental Design

Yield evaluation trials and intentional exposure trials presented in this guide require understanding of the randomized complete block design (RCBD), completely randomized design (CRD) and multi-locations trials.

Randomized complete block design (RCBD)

The use of a randomized complete block design (RCBD) with four repetitions is recommended for most standard evaluation trials which involve yield assessment. In the RCBD design, all treatments (advanced clones/varieties) are grouped into uniform blocks of equal size. The main purpose of blocking is to reduce experimental error by eliminating sources of heterogeneity such as soil fertility or field slopes. With a predictable pattern of field variability, plot shape and block orientation can be carefully chosen so that the experimental conditions within each block are as uniform as possible. When the pattern of field variability is unidirectional, long and narrow blocks should be used. When the pattern of variability is not predictable, blocks should be as square as possible.

The randomization process for a RCBD design is applied to each of the blocks. Randomizing can be done with a table of random numbers, by drawing lots or with the use of MS Excel.

Analysis of Variance (ANOVA) is used to analyze the data collected in a RCBD. The three sources of variability used in the statistical model are the treatment (variety/potato clone), the blocks (repetition) and the experimental error.

Completely randomized design (CRD)

The completely randomized design (CRD) is considered appropriate for trials with homogeneous experimental units where environmental effects are relatively easy to control, such as in greenhouses and other controlled environments. This design is mostly used to evaluate clone response to the pathogen during the intentional exposure trials when the pathogen distribution is random and does not follow any distribution pattern due to field slope, neighbor crops, water irrigation, etc. In a completely randomized design, the treatments are assigned completely at random so that each experimental unit has the same chance of receiving any one treatment.

The randomization process for a CRB design is applied to the whole trial. Randomizing can be done with a table of random numbers, by drawing lots, or by using MS Excel.

Analysis of Variance (ANOVA) is used to analyze the data collected in a CRD. Only two sources of variability are used in this statistical model: the treatment (variety/potato clone) and the experimental error.

Multi-locational variety trials (MLVT)

Multi-locational variety trials (MLVT) are generally conducted to confirm the performance and evaluate the stability or general adaptation of clones that have already shown promise in smaller-scale trials. MLVTs consist of a series of 4 or more similar trials, usually RCBD, which include the same treatments (genotypes) and controls. The trials are usually conducted in locations (environments) that represent the range of production conditions envisioned for a candidate variety.

A combined Analysis of Variance over the sites can be used only after testing the homogeneity of experimental variance across the sites. Experiments with high coefficients of variation are usually eliminated from the analysis. The sources of variability used in the statistical model are the environment (E) or site, the blocks within each environment, the treatment (variety/potato clone) or genotype (G), the site-treatment interaction --also called GxE (genotype x environment) interaction-- and the experimental error.

A deeper analysis of MLTV through Additive Main effect and Multiplicative Interaction analysis (AMMI) (Ebdon and Gauch, 2002; Varela *et al.*, 2006) or GGE bi-plot analysis (Yan, 2002) will provide more precise information about the overall performance of the cultivars, the environments, and the behavior of the cultivars over the range of environments.

Others

The balanced incomplete block design (described in the annex 1) is also used in assessing post-harvest quality of clones using a panel of evaluators.

Depending on the type of trial and its objectives, additional trial designs (such as a split-plot design and lattice design) can be applied. Brief descriptions and the randomization processes of those designs are provided in the annex 1. Further information can also be obtained from technical manuals dealing with experimental trial designs (Gomez and Gomez, 1984).

Field Management

Field management is dependent on the type of trial. A yield trial will preferably be managed according to standard commercial practices. However, resistance trials will demand intentional exposure, high pathogen pressure and limited use of control

measures. In each chapter, recommended field management practices will be described.

Recording and analyzing data

Data recording

This manual is accompanied by a field book designed in MS Excel which specifically deals with each trial.

The first three worksheets of the field book are for recording information about the trial location, description and management, and the plant materials used. Two other worksheets are provided to collect data relative to pest monitoring and climate. Additional worksheets are specific to each chapter of the guide and should be used for collection of the trial data. Some of these worksheets contain formulas that assist with the computation of complementary variables.

The datasheets facilitate the storage of performance data within cooperating institutions and, being standardized, can be easily uploaded in CIPPEX. This software, developed by CIP, facilitates storage, analysis, reporting, and sharing of data between CIP and cooperating institutions.

Data analysis

By using some initial simple statistics to understand the type of data collected during the experiment, entries to be excluded from the analysis can be identified and appropriate for data analysis can be defined.

Measurements such as weight, size and counting are quantitative continuous/discrete variables and are analyzed using parametric statistics when the data distribution is normal. However, numerous data cannot be measured; they are ranked or attached to a rating scale. For example, qualitative ordinal variables are used to characterize the taste, the flavor or the appearance of a clone. The percent of plant infection --which is used in evaluating clone resistance to a disease-- is also a quantitative ordinal (pseudo- quantitative) variable. This variable, which represents the evaluator's estimation of the damage, is more of a ranking than a measurement. Ordinal variables are analyzed and compared using non-parametric methods of analysis.

Each chapter in this guide will provide the basic guidelines for data analysis and interpretation.

Data should be analyzed with the appropriate statistical program (available in CIPSTAT[1]). CIP´s Research Informatics Unit (RIU) can provide assistance with these programs.

(1) http://research.cip.cgiar.org/cipstat/index.php

Assessing potato clone tuber yield

Conducting yield evaluation trials

Location

- Season 1: During the first season, yield trials are established in a location representative of the targeted production area. However, the number and quality of the seed used might force this first evaluation to be located on the secure ground of the research center.
- Season 2: Yield trials are established in one or more locations representative of the targeted production area.
- Season 3: Yield trials are established in four or more locations representative of the targeted production area to evaluate the stability or adaptability of the most promising clones identified in previous seasons.

Materials: clones, control varieties, seed

Clones or varieties from CIP and/or local breeding programs can be evaluated.

At least two of the most commonly-used varieties should be used as controls. If processing performance is to be determined, at least one locally-important chipping/French fry variety should be included in the trial for comparison.

High-quality seed of the same origin should be used as control varieties and clones. The tuber yield trial in the first season requires at least 40 seed tubers per entry, to be planted in four replications in one location. During the following seasons, the plot size and number of locations should be increased depending on seed availability.

- Season 1: 40 seed/clone
- Season 2: 240-400 seed/clone
- Season 3: >400 seed/clone

Experimental design

To evaluate advanced clonal material, it is necessary to incorporate a minimum number of replications in the trial design. The use of a randomized complete block

design (RCBD) with a minimum of four replications is recommended for the standard evaluation trials.

Rectangular plots of double or multiple rows are preferable to long, single row plots. Other experimental designs, such as the completely randomized design (CRD), split-plot design, and incomplete block design, can also be used depending on the conditions of the experiment.

Multi-location trials are usually conducted during the third season, when sufficient seed is available. In each location, a separate replicated trial is conducted using a common design. Common selected clones and controls are used in the different locations. Special attention should be given to the quality of the seed used for the multi-location trials: only healthy seed should be used in order to avoid the spreading of seed-born diseases.

Field management

Standard agronomic practices and local procedures to protect the seed plot from pests, diseases and virus vectors are applied.

Evaluation parameters

Once the trials have been established, the following data should be collected during the growing season:

- Number of plants/plot: this data is collected 45 days after planting.
- Plant habit: this data is collected 45 days after planting.
- Plant vigor: this data is collected 45 days after planting.
- Flowering stage: this data is collected 60 days after planting.
- Senescence stage: this data is collected 90 days after planting.

Plant habit and flowering and senescence stages are measured using scales described by Gomez (2006). Plant vigor can be evaluated using a scale from 1 to 9 where "1" is assigned to least vigorous plants and "9" describes very vigorous plants.

During this time, observations on disease and pest damage can also be recorded. With material resistant to late blight, the percentage of late blight infested plants can be measured. Because the purpose of the trial is to evaluate yield under optimum crop management, fungicides should be used against severe late blight attack

in order to protect the crop. Specific evaluation of clone resistance to local pests and diseases should be made using intentional exposure trials, which are described in the following chapters.

At harvest, tubers are graded into 3 categories. The weights and numbers of tubers in each category are recorded.

In CIP, the following categories are used:

- Category I: commercial
  Tubers weighing 200-300 g or measuring >60 mm
- Category II: commercial
  Tubers weighing 80-200 g or measuring 30-60 mm
- Category III: non-commercial
  Tubers weighing < 80 g or measuring < 30 mm
  Tubers with external defects

These categories are arbitrary and vary according to the country and region in which they are being evaluated. Each evaluator is free to use locally-relevant criteria; however, each category should be precisely defined in order to facilitate comparison of data between countries.

Internal defects should be reported at harvest time. This is critical for estimating processing quality. A sample of 10 commercial tubers should be cut transversally and checked for external defects such as cracking, secondary growth and warts, and internal problems such as hollow heart, black spots, heat necrosis, and rot. For each entry, the number of affected tubers is recorded on the tuber yield datasheet. Percentage of affected tubers is calculated.

Note: Tubers harvested from the tuber yield trial may be used for subsequent evaluation of post-harvest quality, processing and cooking performance, and storability.

Recording and analyzing data

Data recording

All collected data are registered in the tuber yield datasheet.

In order to facilitate comparisons between trials and locations, data for yield assessment (such as total weight or commercial weight) should be expressed in tons/ha;

therefore data about harvested plot size should carefully be reported in the trial information worksheet.

Data analysis

Simple statistics such as mean, standard error, frequency distribution and boxplots should be used to explore the data.

Yield data are analyzed using variance analysis (ANOVA) and means are compared using statistical comparison tests such as LSD, Tukey, Waller-Duncan, and Bonferroni. Orthogonal contrasts and Dunnett tests can be used to compare the advanced clones with the control(s).

The analysis of residuals is recommended to test the validity of the model and to analyze the behavior of the variance (homogeneous or not).

All analysis can be performed using R or other statistical packages. CIPSTAT, which uses the R package, facilitates analysis and reports of the results.

Data interpretation

Validation of the experiment

An experimental trial for tuber yield evaluation is considered to have been carried out under appropriate conditions if the experiment's coefficient of variation does not exceed 30%.

Selection criteria

Performance of each advanced clone is compared with the performance of the control(s). It is important to consider the commercial yield of the entry rather than the total yield. In most situations, the ability of a clone to develop numerous small tubers will be viewed as a negative characteristic.

Assessing the post-harvest quality and storability of potatoes

The primary objective of evaluating post-harvest traits is to obtain information about the potential of advanced clones for diverse end uses, ranging from fresh consumption to processing. These evaluations provide important information to guide potato breeding and selection programs, and the results may be useful for recommending new varieties for specific uses.

This protocol details procedures for determining:

- Dry matter content and specific gravity
- Chipping performance
- French-frying performance
- Cooking quality, including texture and flavor components
- Storage behavior, including sprouting and weight loss as well as diseases and rotting

Conducting post-harvest evaluation

Post-harvest and storability characteristics can be evaluated using healthy tubers harvested from tuber yield trials.

Conditions:

Environmental factors (principally temperature fluctuations, rainfall, altitude, and soil fertility), management and genotype x environment interaction influence post-harvest performance. Site, weather, and management conditions of the production materials should therefore be recorded on the appropriate field book datasheet.

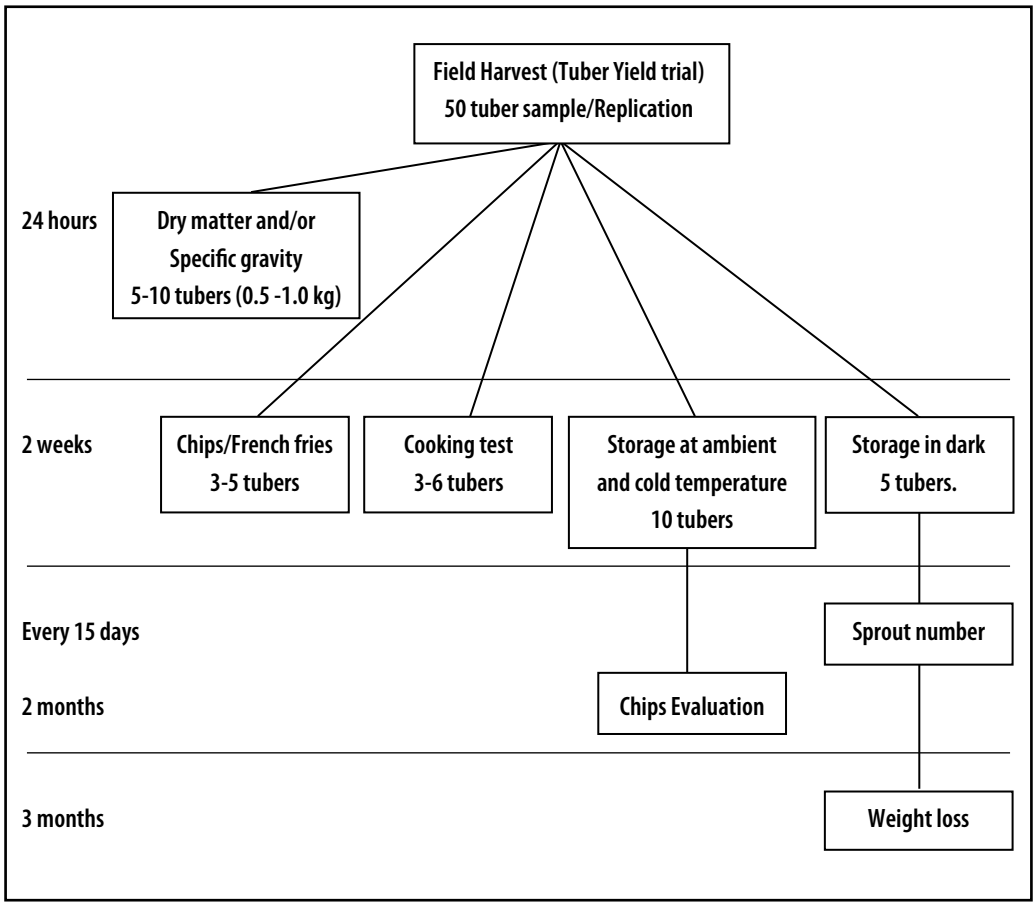
To determine varietal stability of traits, evaluations should be conducted on material produced in contrasting environments (for instance, in two sites or multi-locational trials), particularly if altitude or season is relevant to the particular ecology of the area being targeted for variety development.

Materials: clones, control varieties

In each replication, samples are taken of one or more healthy tubers of commercial size per plant until obtaining a minimum of 25 tubers per clone. The sampled tubers should be uniform and representative of the clone's size and shape. If processing quality is to be determined, at least one locally important chipping/French fry variety should be included in the trial for comparison.

The recommended number of necessary tubers and the timing of the test evaluations are shown as follows:

Flow diagram: Post harvest evaluations



Assessing solid content of tubers

Materials

If possible, dry matter content should be measured within 24 hours after harvest to avoid post-harvest changes due to shrinkage. Tubers should be free of disease and undamaged. Peeling is not necessary.

The measure of all parameters requires a balance accurate to 0.1 g.

Evaluation parameters

Dry matter content

In addition to the balance, an oven is needed to determine dry matter content.

Step 1: Chop five tubers (about 500 g total) into small 1-2 cm cubes, mix thoroughly, and take two sub-samples of 200 g each. It is important to sample all parts of the tuber, because dry matter content is not uniform throughout the tuber. Determine the exact weight of each sub-sample and record it as fresh weight.

Step 2: Place each sub-sample in an open container or paper bag and put in an oven at 80˚C for 72 hours or, after checking sample weight at regular intervals, until constant dry weight is reached. Weigh each sub-sample immediately and record as dry weight.

Step 3: Calculate the percent dry matter content for each sub-sample with the following formula:

dry matter = (dry weight / fresh weight) x 100

Step 4: Calculate the mean dry matter of the two sub-samples.

Specific gravity

Specific gravity can also be used to indirectly evaluate the dry matter content of one clone. Two methods can be used for determining tuber specific gravity: the weight in air/weight in water method (which is more accurate) and the hydrometer method.

Determining specific gravity with the weight-in-air/weight-in-water method
This method requires the use of a scale equipped with a hook underneath in order to hold a basket, which will be immersed in water.

The weight-in-air/weight-in-water method can be performed as follows:

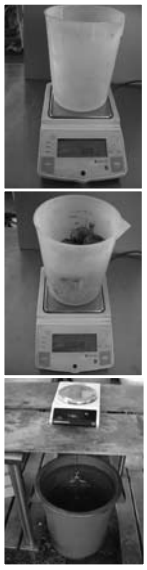
Step 1: Place a plastic beaker or metal basket on a balance, and re-calibrate the balance to zero.

Step 2: Place 4 kg of commercial size potatoes in the basket and weigh; record data as weight in air.

Step 3: Immerse the plastic beaker or metal basket and potatoes in water and weigh again; record data as weight in water.

Step 4: Calculate specific gravity with the following formula:

Specific gravity = (weight in air) / (weight in air – weight in water)



Determining specific gravity with the hydrometer method

If a calibrated hydrometer is available, it can be used as follows:

Step 1: Place a plastic beaker or metal basket on a balance, and re-calibrate the balance to zero.

Step 2: Place the exact weight of 3629 grams of commercial size potatoes in the beaker or basket (one potato will have to be cut in order to obtain this exact weight). The 3629 grams is a standard amount to be used if the hydrometer is calibrated (nevertheless, check the manufacturer's manual for confirmation).

Step 3: Immerse a metal basket and the potatoes in water and connect it to the hydrometer. The specific gravity can be read directly from the hydrometer.

Note: Always be sure that the hydrometer is calibrated (for more detailed information, consult the manufacturer's manual).

Data recording

The "Assessment for Post-harvest Quality: Dry Matter-specific Gravity datasheet" (Post-harvest quality 1 worksheet) should be used to record and compute data.

Data analysis

The data, dry matter percentage and/or specific gravity can be analyzed according to the design (entry, repetition) used in the field when the samples are taken from each experimental unit of the field trial.

Simple statistics such as mean, standard error, frequency distribution and boxplots should be used to explore the data.

Continuous data expressed in percentage (such as percentage dry matter) do not need to be transformed before the analysis.

Data are analyzed using R or other statistical packages. Dry matter percentage and/or specific gravity data are analyzed using variance analysis and means are compared using LSD, Tukey, Waller- Duncan, Bonferroni and/or other tests. Orthogonal contrast is used to compare the clones with the local control (Dunnett test).

CIPSTAT, which uses the R package, facilitates analysis and reports of the results.

The analysis of residuals is recommended to test the validity of the model and to analyze the behavior of the variance (whether it is homogeneous or not).

Data interpretation

Percent dry matter and specific gravity are highly correlated and are two alternative means of estimating the solid content of tubers. Both variables give an indication of processing and cooking quality.

Selection criteria

In general, dry matter content of more than 20% and a specific gravity of 1.080 or greater are considered acceptable. These values correspond to a solid content of

about 18%. Tubers meeting these criteria produce high yields of chips that absorb less oil and have better texture than chips made from potatoes with lower solids. Lower values than these indicate unacceptable quality for most processing purposes.

Assessing chipping performance

Materials

Harvested tubers should rest ("stabilize") at room temperature for 10 days before evaluating the chipping performance.

Evaluation parameters

Variables to be measured are the degree of darkening that occurs during frying and the amount of oil absorbed in the process.

Chip darkening

Tubers should be free of disease and undamaged. Peeling is not necessary. Each sample comprises six tubers.

The Potato Chip Standard Color Chart is used to assess the degree of darkening.

Step 1: Cut each tuber perpendicular to the long axis and take three 0.5 mm slices from the central part of each half.
Step 2: Rinse the slices in water, shake the water off the slices and allow the surfaces to dry.
Step 3: Fry the slices in oil at 176-180°C until the oil stops bubbling (approximately 3mn).
Step 4: Evaluate the color of the chips with the Potato Chips Standard Color Chart on a 1-5 scale where 1 is light cream or yellow and 5 is dark brown.

Oil absorption

In addition to the innate or physical characteristics of a variety (for example, specific gravity), several other factors affect oil content, so it is important to use uniform procedures.

Determining oil absorption rate with the press method

This method requires a carver press, a balance and paper towels.

Step 1: Grind 20 to 30 g of fresh, cooled potato chips in a mortar and pestle or other suitable apparatus and thoroughly mix the sample.

Step 2: Place paper towels in the bottom of the sample chamber to absorb the expressed oil.

Step 3: Weigh a sample of about 10 g of the ground chips (record the data as initial weight) and place it in the sample chamber.

Step 4: Place the piston portion of the assembly on the sample and chamber.

Step 5: Place the entire assembly in the press and pump it up at a rate of one stroke per two seconds until the pressure reaches 15,000 pounds/inch2 (psi).

Step 6: Allow 20 seconds for the pressure in the press to drop off and then pump it back up to 15,000 psi.

Step 7: Set the timer for three minutes.

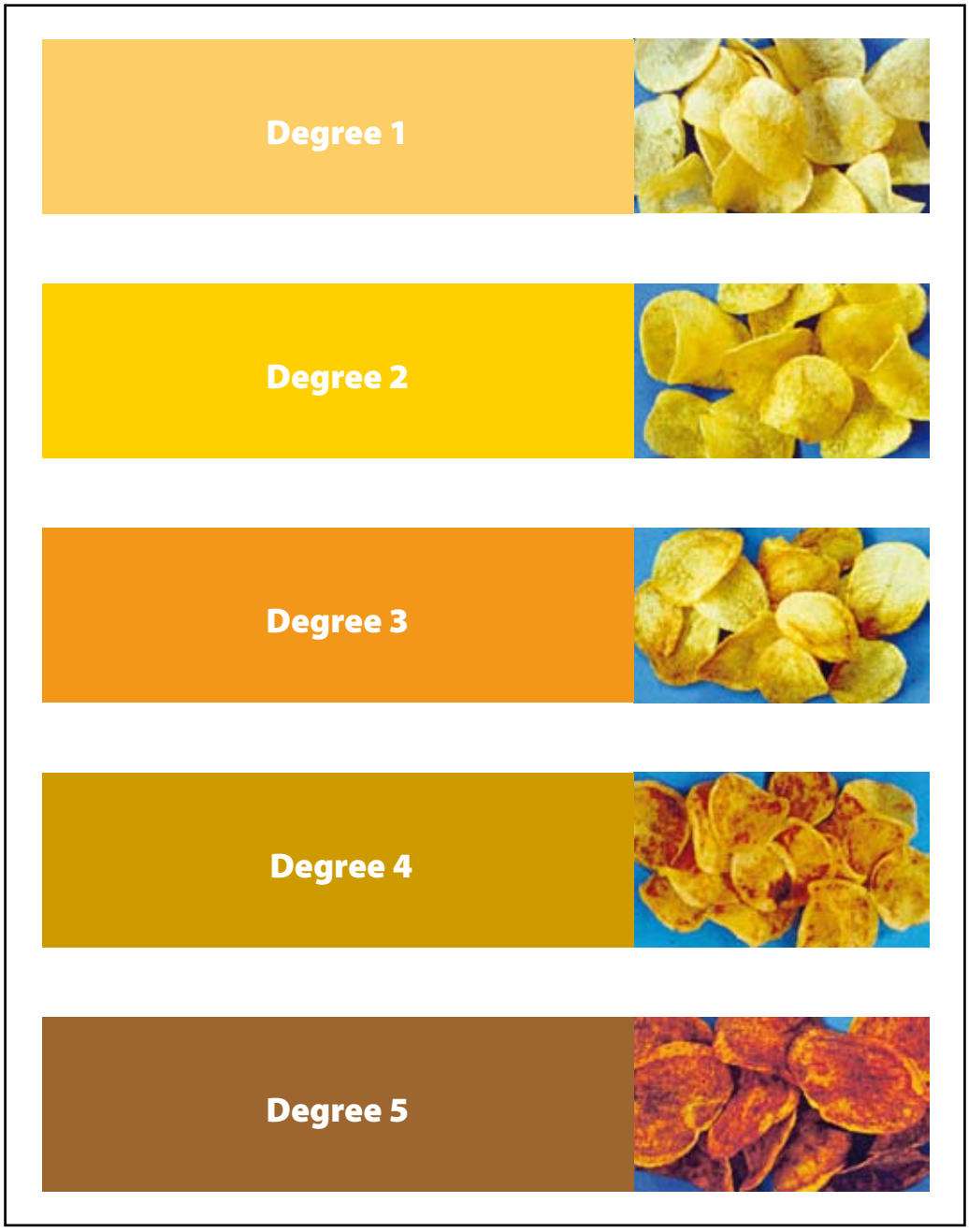
Step 8: At the end of three minutes, release the pressure and remove the sample cake from the chamber. Take great care to not to leave any of the cake behind and not to take any oil with the cake.

Step 9: Weigh the sample cake (record the data as final weight).

Step 10: Determine oil content in a 10g sample with the following formula:

Oil absorption = initial weight - final weight

Potato Chips Standard Color Chart



Data recording

The "Post-harvest quality evaluation: Chipping, French-frying and cooking data-sheet" (Post-harvest quality 2 worksheet) should be used to record and compute data for both chip darkening and oil absorption.

Data analysis

Chip darkening data are considered quantitative ordinal data and are analyzed with nonparametric analysis. Values of entries are compared using the Friedman, Durbin or Kruskal Wallis tests (Conover, 1999).

The variable "absorbed oil in a 10 g sample" is analyzed using variance analysis (ANOVA) and means are compared using statistical comparison tests such as LSD, Tukey or Waller-Duncan. Orthogonal contrasts (obtained by using the Dunnett test) can be used to compare the performance of the clone with the performance of the control(s).

These analyses can be performed using R or other statistical packages. CIPSTAT, which uses the R package, gives analysis and reports on the results.

Selection criteria

Light colored chips are preferred, with degree of darkening up to 3 usually accepted by the industry.

Oil used in the manufacture of potato chips or other fried potato products may be one of the most costly ingredients. Excessive oiliness in fried products indicates not only a poor quality product, but also the loss of an expensive ingredient. Excessive oil results in greasy or oily chips-- traits that are undesirable to the consumer. Oil absorption greater than 40% is considered unacceptable.

Assessing processing and cooking performance

Conditions of the evaluation

Evaluations of French-frying and the cooking performance of potatoes require sensory perceptions of panels of tasters.

Materials

Tasters

The reliability of the information obtained depends on the evaluators' abilities to recognize differences among the samples they are evaluating. Tasters must therefore have no major physical handicaps that may hinder their ability to recognize organoleptic traits, and must not have any type of respiratory infection (such as flu) that may impair the senses. A simple taste test of solutions of sugar, salt, citric acid and acetic acid in different concentrations can help to screen candidates.

Tasters should be trained, and tasting should be conducted taking into account the following most common psychological errors that occur during sensory evaluation:

- Habituation error- the evaluator continues to give the same answers when samples are presented in increasing or decreasing order.
- Expectation error- the anxious taster finds a difference where there is none.
- Induced error- the evaluator knows how to do the test, and external factors suggest differences where there actually are none.
- Benignity error- the evaluator is a friend of the researcher and, for that reason, gives the sample a higher assessment.
- Central tendency error- the evaluator hesitates to use the extreme values of the scale.
- Contrast error- a bad or disagreeable sample is presented after a good or agreeable sample.
- Sample position error- different samples are assessed in the same way because they have been presented in the same order.
- Association error- a tendency to repeat previous impressions.

In sensorial or food evaluations, the most important errors are central tendency, sample position, and contrast.

Experimental design

Since the same taster should not evaluate more than 10 samples, the evaluation will be organized according to a balanced incomplete block design (BIBD). Each sample will be evaluated the same number of times and each taster will evaluate a different set of clones. The list of samples to be evaluated by each taster must be defined and reported on the French fry score sheet before initiation of the experiment. Indication to set the design is provided in annex 1.

Management of the evaluation

The following rules should be applied during the evaluation:

- Before the evaluations are conducted, a technician must clearly explain the procedures, including how to record the data, to the tasters.
- Tasting should be conducted in an odor-free room, preferably between 10 a.m. and 2 p.m.
- Panelists should not eat or smoke for at least one hour before the evaluations.
- Panelists should not be expected to evaluate more than about 10 samples in any tasting session.
- Samples should not be swallowed and a cup of water should be available to all panelists to rinse their mouths between samples.
- Samples should be maintained at the correct temperature.
- Names or other known identifiers of the varieties to be tasted should be concealed and a code should be assigned to each entry.
- Panelists should not discuss their evaluations until the session is finished.
- No one person should have the sole responsibility for judging a sensory characteristic; the combined judgment of several people will minimize any individual sensitivity variation or fluctuation.

Evaluation parameters

French fry performance

This test is designed to simulate the commercial process used to manufacture French fries. A tasting panel comprised of four trained tasters evaluates the French fries.

Each sample includes three tubers, free of disease and undamaged.

Step 1: Slice the tubers into strips 1 cm in cross-section. Take slices from three different parts of the tuber: four each from the center, the outer part and the intermediate region of each tuber (3 x 3 x 4 = 36 slices).
Step 2: Assemble four tasting samples (one for each panel member) consisting of randomly chosen slices from each of the three parts from each of the three tubers, a total of nine strips per panel member.
Step 3: Pan fry in cooking oil at 193˚C for 1 minute. Drain off excess oil and leave to cool for about 1-2 minutes.
Step 4: Continue frying at 193˚C for 1½ minutes.
Step 5: Sort strips into inside and outside slices. Present the samples to panel members and evaluate according to external appearance, color, internal color and texture (mealiness) of the flesh.

Note: Good dry matter (and hence good texture) is not uniform throughout the tuber and is often limited to the center of the tuber.

Cooking quality

A tasting panel comprised of 6-12 trained tasters evaluates the cooking/eating quality of boiled or microwaved tubers. Local varieties with good and poor cooking quality should be used as controls.

Each sample includes 3-6 tubers, approximately 7.5 cm in diameter, free of disease, undamaged and washed free of soil and debris.

Step 1: Wrap each tuber in a damp paper towel and cook in a microwave oven (700-1000 W) at full power for 10½ minutes, or place tubers in boiling water until a pin/probe penetrates the tissue.
Step 2: Record the average time needed for cooking the stem and bud ends on the "Post-harvest quality evaluation: Chipping, French-frying, and cooking datasheet" (Post-harvest quality 2 worksheet).
Step 3: Immediately after cooking, wrap the potatoes in thin aluminum foil to keep hot until they are presented to the panel.
Step 4: Cut one tuber in half for each panelist and immediately proceed with evaluation of color, texture and flavor.

Data recording

Tasters record their scores on the individual French fry score sheet (annex 3) and/or the cooking quality score sheet (annex 4).

Data are then processed. Each mark from the individual score sheet is converted into a weighted number, with higher weight given to characteristics reflecting the best quality. A suggestion of the weight is given in each score sheet (hidden column). The panelists' overall impressions are computed by adding all the weighted numbers and are then recorded in the "Post-harvest quality evaluation: Chipping, French-frying, and cooking datasheet" (Post-harvest quality 2 worksheet).

Data analysis

Overall French fry and cooking quality rating data are analyzed as nonparametric variables in a Balanced Incomplete Block Design. The Durbin test can be used to compare the performance of each entry.

Additional characteristics such as texture and flavor can be correlated with dry matter content.

Selection criteria

The Durbin test allows for the identification of genotypes with best processing quality for French fries and/or cooking.

Evaluating storage behavior

Conditions: storage

Material is stored in a cool, dark, ventilated room.

Materials

Tubers harvested from the tuber yield trials are used to determine storage behavior. Only healthy and clean (free of excess soil and debris, but not washed) tubers will be stored and evaluated.

Evaluation variables

Sprouting and weight loss

Step 1: Weigh a sample of 10 commercial tubers in good condition for each entry and record initial weight.
Step 2: Keep the tubers in containers at room temperature in dark, ventilated storage.
Step 3: 45 days after harvest, for each entry withdraw five tubers at random and record the sprouting degree per tuber. Replace the tubers carefully into the container after examination.
Step 4: 90 days after harvest, for each entry withdraw five tubers at random and record the sprouting degree per tuber.
Step 5: 90 days after harvest, de-sprout the tubers and weigh the sprouts and tubers separately.

Diseases and rotting

At the end of the three-month storage period, the level of disease and rotting is assessed by recording the numbers of tubers affected and any other observations.

Data recording

The overall storage conditions should be registered in section 10 of the "Climate data worksheet" of the ICG field book.

All the results and observations are registered on the "Assessment of storability datasheet" (Post-harvest quality 3 worksheet).

Data analysis

Final weight is analyzed with an analysis of covariance (AVCOVA) taking into account the weight before storage. Comparative tests on the covariance adjusted means will be used to compare the storage behavior of the clones.

Selection criteria

Results will allow characterization of the clones' storability and comparison with local controls. Varieties with long dormancy period and minimum weight loss will be preferred for processing.

Assessing potato clone field resistance to late blight

Introduction

Phytophthora infestans, causal agent of late blight

Late blight is a polycyclic disease, caused by *Phytophthora infestans*. A recent classification scheme based on molecular analysis of the nuclear srRNA gene as well as ultra-structural data includes the genus *Phytophthora* into the kingdom Chromista together with golden and brown algae and diatoms. The genus *Phytophthora* occurs within the phylum Oomycota, which is characterized by zoospores propelled by heterokont (of unequal length) flagella and have cellulose in their cell walls. It is now clear that oomycete fungi are not related to the ascomycete and basidiomycete true fungi (Dick, 1995).

Mycelium of *P. infestans* cannot survive in the absence of a living host cell whereas sporangia may survive days or weeks in soil. The asexual form of *P. infestans* is essentially an obligate parasite, requires a living host (crop debris or solanaceous weeds) for long-term survival and cannot overwinter or overseason. However, in locations where sexual reproduction occurs, the resulting oospore can survive for months or years in the absence of living hosts (Drenth *et al.*, 1995).

Recent *P. infestans* migrations

After a very long time during which *P. infestans* populations were considered asexual, a 1984 report of A2 mating types in Western Europe was the first indication of new and dramatic developments in the pathogen populations (Hohl and Iselin, 1984). The analyses of a large number of dispersed local populations indicated, surprisingly, that the changes were not restricted to Western Europe, but were worldwide (Fry *et al.*, 1993; Goodwin and Sujkowski, 1995). The genetic diversity generated by sexual reproduction can lead to more aggressive genotypes.

Late blight

Late blight is a polycyclic disease, having several cycles of infection and inoculum production during one growing season. Thus, the infection is expected to increase proportionally to both the initial amount of inoculum and the amount of new inoculum produced during the growing season. The amount of inoculum produced depends on the host, pathogen, environment and management conditions.

Late blight symptoms

Late blight symptoms in leaves

The disease appears first as water-soaked irregular pale green lesions mostly near tips and margins of leaves. These lesions rapidly grow into large brown to purplish black necrotic spots (Picture 1). During morning hours, a white mildew consisting of sporangia and spores of the pathogen can be seen on the lower surface of infected leaves, especially around the edges of the necrotic lesions. Thus the symptoms can vary, depending on the age of the lesion, the environmental conditions prior to observation and the tissue infected. Very young lesions are small (2-10 mm) and irregularly shaped, and may be surrounded by a small halo (collapsed, but green tissue bordering the dark necrotic lesion). As lesions grow, they become more circular until they are limited by the leaflet margins. They are usually not delimited by the veins, and older lesions are typically surrounded by a chlorotic halo.

Late blight symptoms in stems

When late blight attacks the stem, it can cause girdling and leaf wilting above the point of infection. Light to dark brown lesions on stems or petioles elongate and encircle the stems (Picture 2). Stem lesions become brittle and the stem frequently breaks at that point (Picture 3).

Late blight symptoms in tubers

Infected tubers show irregular reddish-brown to purplish slightly depressed areas that extend deep into internal tissue of the tubers (Picture 4). The infected tubers are initially hard, dry and firm but may be invaded by other pathogens, mainly bacteria, leading to soft rot. A pungent, putrid smell is often associated with heavily infected fields. This is due to rotting of dead tissue and is not a direct consequence of late blight.

Resistance to Late Blight

Until recently, there has been general consensus that most resistance to P. infestans could be classified into two types. The first is governed by single dominant genes with major effects and a clear, discontinuous segregation of progeny; the second is governed by several or many genes, called minor genes, with small cumulative effects and continuous distribution of resistant genotypes. Major gene resistance has also been described as vertical resistance, R-gene resistance, qualitative resis-

Picture 1. Late blight symptoms on potato leaves

Picture 2. Late blight symptoms on potato stem

Picture 3. Late blight symptoms on potato stem

Picture 4. Late blight symptoms in potato tuber

tance, specific resistance, race-specific resistance, unstable resistance, and complete resistance. Minor gene resistance has been described with contrasting names, such as horizontal resistance, polygenic resistance, quantitative resistance, general resistance, nonrace specific resistance, stable resistance, partial resistance, field resistance and rate-reducing resistance.

Conducting late blight intentional exposure evaluation trials

Locations: screening sites

The environmental conditions at screening sites must be conducive to late blight. Knowledge about the pathogen isolates from the screening sites is useful for subsequent disease management considerations.

Materials: clones, control varieties, seed

Clones or varieties from local breeding programs or from CIP can be evaluated.

Controls: A small number (1-5) of potato genotypes (clones or varieties) with known levels of resistance ranging from susceptible to highly resistant should be included in the evaluation trial. CIP currently uses the very susceptible Peruvian variety Chata blanca (CIP 701209), the susceptible Peruvian variety Yungay (CIP 720064), the moderately resistant Colombian variety Montserrate (CIP 720071), the resistant to moderately resistant Mexican variety Atzimba (CIP 720045) with mayor genes and the CIP resistant clone LBr-40 (CIP 387164.4) as controls.

Seeds: Uniform healthy tubers of the same origin for both advanced clones and controls should be used. Late blight evaluation trials require 40 tubers, although a minimum of 20 tubers of each clone can be used during the first year of evaluation.

Experimental design

The evaluation of advanced clones should be done in a replicated trial using 4 repetitions with a recommended 10 tubers per repetition.
A completely randomized design is preferable when resistance to late blight is the only factor of evaluation in the trial. However, the evaluation is often combined with yield evaluation and, in this case, a complete block design is preferred

Field disease variability

Different measures can be taken to reduce or control variability of disease severity in the field. One measure is inoculation, which is especially useful in areas where natural inoculum may not be very high. Protocols for field inoculation are available in the literature (Forbes et al, 1993).

Another measure frequently used is to plant one susceptible or both a susceptible and a moderately resistant genotype of potato at regular intervals within the trial in order to produce continuous sources of inoculum. These additional genotypes are often referred to as "spreader rows". Spreader rows may introduce other biases. If the genotype used as a spreader is very susceptible, it will make its immediate neighbors look more susceptible due to the large amount of inoculum the spreader sheds. To avoid this, spreaders can be planted between each clone so that all clones receive an equal amount of inoculum, but this doubles the size of the experiment. If the resistance of a particular plant genotype is based primarily on sporulation, the use of heavily sporulating spreader rows can lead to an underestimation of the resistance of this genotype.

Field management: protecting plants with fungicides

Late blight occurs very early in many tropical and subtropical locations. If infections occur when plants are very small, differences between resistant and susceptible genotypes may be difficult to appreciate. Under this condition, it is advisable to protect plants with fungicides until they are of sufficient size for evaluation. Generally, a contact fungicide is applied until the plants are considered large enough for evaluation, i.e. when they reach 30% of their full-grown leaf area.

Evaluation parameters

Measurements of plant development (vigor) are generally recorded in addition to disease severity.

Readings of percentage of infected area

The percentage of leaf infection is recorded throughout the season and the date of each reading is carefully noted in the section 5 of the Pest monitoring datasheet of the ICG field book. Data are collected on each experimental unit (each clone or variety within each replication).

Disease readings are taken on the basis of the percentage of leaf area affected by late blight. The first reading is taken 30 to 40 days after planting and 10 days after the last fungicide application. It is important to begin readings as soon as symptoms appear, so the field should be carefully observed to detect the initiation of disease.

Because the percentages of leaf area are used to compute the Area Under the Disease Progress Curve (AUDPC), the frequency of readings is not really crucial. If the disease is advancing quickly in susceptible genotypes, readings should be done frequently (every 7 days). If the disease is advancing slowly, the interval between readings can be longer (every 14 days). The objective is to have readings at low, medium and high levels of disease in all genotypes, including susceptible ones. One of the strengths of the AUDPC is that readings do not have to follow a regular schedule.

When evaluating late blight, most researchers estimate the percentage of total leaf area that is affected by the disease. This is done by simply comparing the green and nongreen portions (assuming late blight is the only or dominant foliage disease). Thus, one mentally estimates the percent of infected foliage in the plot. This standard procedure works well (Forbes and Korva, 1994), especially when readings are integrated into a measure like the AUDPC.

Source of errors when estimating the percent infection
Estimation of percent infection is nonetheless subject to several sources of error.

1. Underestimation occurs when evaluation is made only on that portion of the disease with visible symptoms that is still on the plant. Infected leaflets eventually fall off and the timing of the fall is probably cultivar-dependent. Diseased tissue can be green and symptomless and therefore overlooked during the evaluation. Infected green tissue can also be sporulating, but this is not always visible unless one is very close to the leaflet. This level of scrutiny is generally not employed in cultivar evaluations.
2. Human error can occur when proportional differences are evaluated. Research has shown that people estimate disease more precisely at lower and higher levels of severity than at intermediate levels. The use of logarithmically-based scales does not necessarily correct this bias.

In general, it is better if all readings for a trial are taken by the same evaluator. It is also best to record readings independently (that is, without knowing the value given at the previous reading) by having someone else register the values in the datasheet or by using a cassette recorder.

Recording and analyzing data

Data recording and computation

Area under the disease progress curve (AUDPC)

Because late blight is a polycyclic disease, CIP recommends the use of the Area Under the Disease Progress Curve (AUDPC) to measure resistance (Fry, 1978). The AUDPC is a resistance measurement calculated from the estimated percentages of leaf area affected recorded at different times during the epidemic.

AUDPC is simple to calculate, uses multiple evaluations, and does not rely on transformations. AUDPC also presents some disadvantages which will be discussed at the end of this chapter.

The AUDPC is frequently calculated using the following formula (Campbell and Madden, 1990):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where "*t*" is the time of each reading, "*y*" is the percent of affected foliage at each reading and "*n*" is the number of readings. The variable "*t*" can represent Julian days, days after planting or days after emergence.

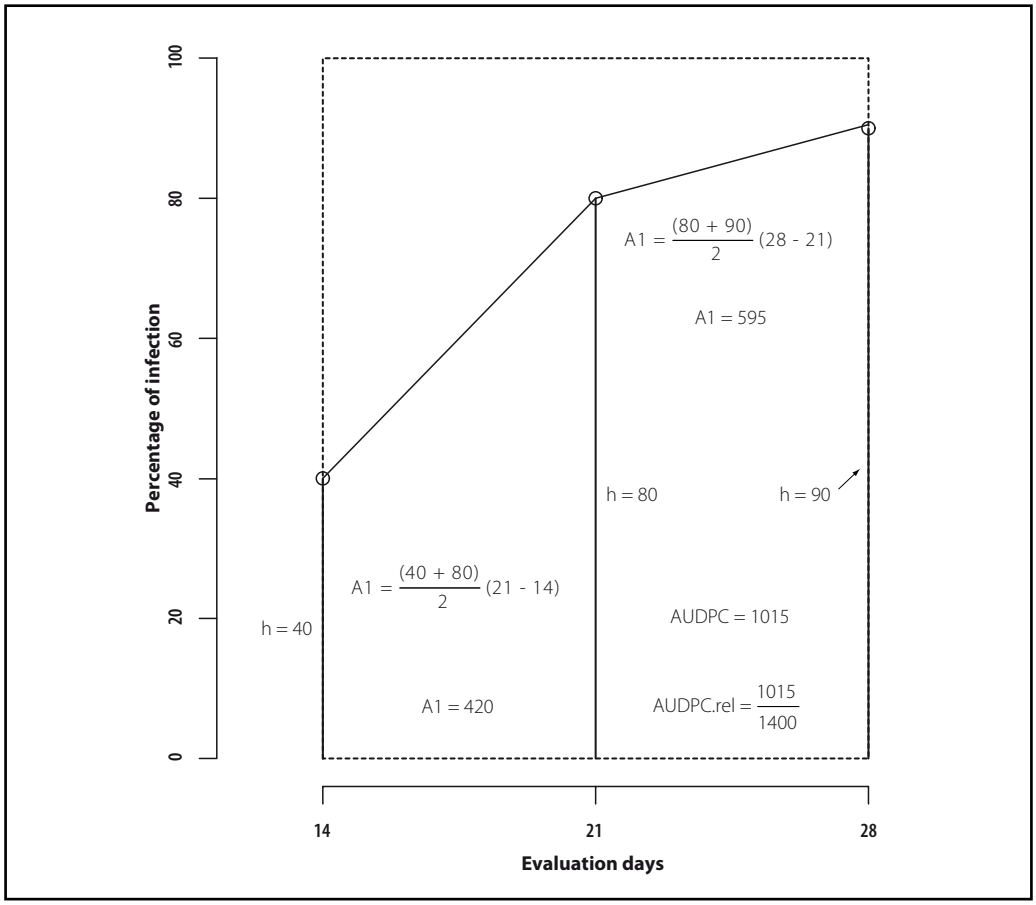
The drawing shown in Figure 1 is a graphic representation of the equation. It shows the AUDPC as a summation of trapezoidal areas.

The computation of AUDPC with Microsoft Excel is presented at the end of the chapter.

The Assessment for Late Blight Resistance datasheet (late blight worksheet) is included in the ICG field book to facilitate the registration of the data and computation of the AUDPC. The dates of each reading are recorded in the Pest Monitoring datasheet and data are used to calculate the intervals between readings.

Note: As mentioned at the end of the chapter, the readings to be included in the AUDPC calculation should be carefully selected when adverse conditions have been present and have prevented good development of the disease.

Figure 1. Graphic representation of the AUDPC.



Data analysis

AUDPC or percent of infection values can be analyzed using variance analysis (ANOVA) after exploration of the data through simple statistics such as means, standard errors, frequency distributions and box plots.

The Dunnett test can be applied to compare the AUDPC or percent of infection means of the evaluated clones with the means of the controls.

The analysis of residuals is recommended to test the validity of the model and analyze the behavior of the variance (homogeneous or not).

The AUDPC and percentage of infection are considered pseudo-quantitative variables with hierarchy and can be analyzed without transformation and compared

using nonparametric methods of analysis with use of Friedman (RCBD) or Kruskall Wallis (RCB) tests.

Nonparametric tests are also useful to compare the resistance behavior of the clones across different trials or in trials in which the coefficient of variation is high (>30%). Those analyses can be performed using R or other statistical packages. CIPSTAT, which uses the R package, facilitates analysis and reports of the results.

If yield has been evaluated in addition to the AUDPC, the correlation between yield and genotype resistance can be calculated with the Spearman method. The Spearman method works by ranking each observation within each replication and the Spearman rank correlation is calculated on the ranking values. A value of the coefficient close to one indicates a good correlation between yield and resistance.

Data interpretation

The AUDPC is a variable which estimates the area under the actual infection curve. It is expressed in % days (that is, the accumulation of daily percent infection values) and is interpreted directly without transformation. The higher the AUDPC, the more susceptible is the clone or variety. It is often helpful to plot the percentage of leaf area infected versus the evaluation date to get a better idea of how clones or varieties perform in the experiment (Figure 2).

Under favorable conditions for the intentional exposure trial, the coefficient of variation of the experiment should not exceed 30%.

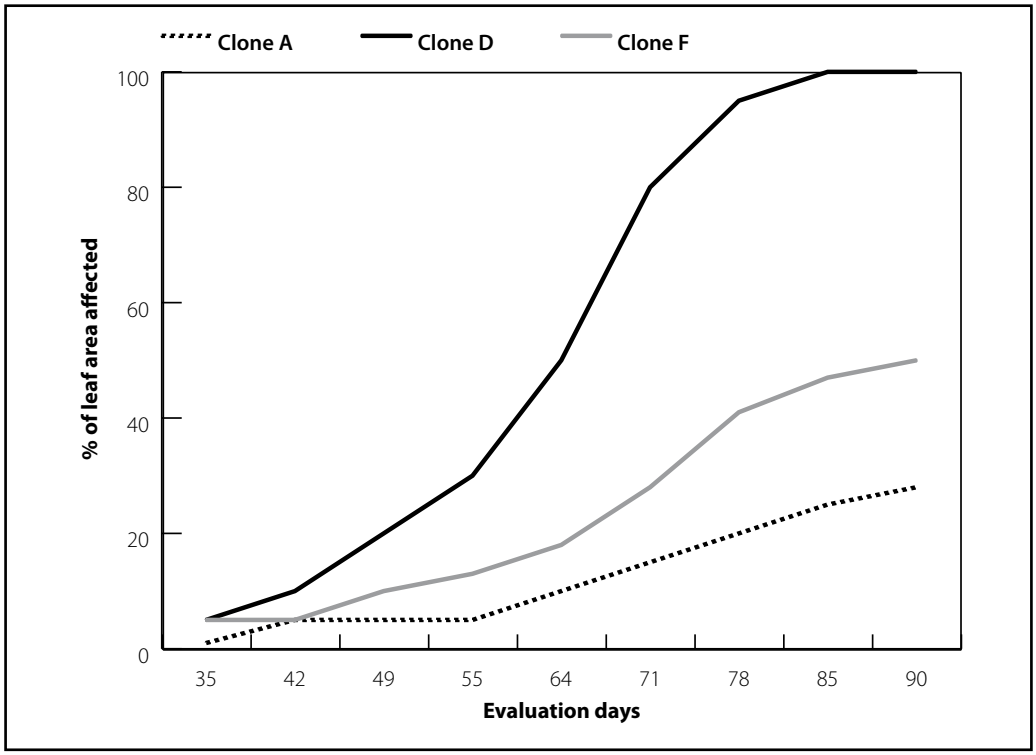
Getting around common problems associated with the AUDPC

Timing the readings

Problem: As mentioned, the AUDPC is robust in the sense that it allows for different intervals between readings. However, the AUDPC can be biased if readings start too long after disease initiation. In this case, part of the disease progress curve of susceptible materials will not be considered in the AUDPC.

In the same way, the AUDPC can be biased if readings are taken too long after susceptible cultivars reach high levels of disease. When this occurs, the difference between resistant and susceptible materials may be underestimated.

Figure 2. Disease severity curves of three potato clones.



Solution: It is important to start the readings when disease begins.

Generally, it is not relevant to continue the readings when the susceptible materials are severely infected. However different AUDPCs may be calculated, one including all materials and a reduced number of readings, and another including more readings but excluding the most susceptible materials.

Handling lack of uniformity of disease

In the field
Problem: The AUDPC is sensitive to lack of disease uniformity in the field.

Solution: Experimental designs for controlling this type of error have been discussed above.

In time
Problem: Adverse conditions have been present and prevent good development of the disease.

Solution: Readings to be included in the AUDPC calculation can be carefully selected. For example, first readings would be excluded from the AUDPC computation when dry weather suddenly stopped the normal spread of the disease.

Dealing with incomparability across experiments

Problem: The AUDPC is generally not comparable across experiments.

Solution: In an effort to standardize the AUDPC, researchers often use the relative AUDPC. The relative AUDPC (AUDPC.rel) is calculated by dividing the AUDPC by the "maximum potential AUDPC."

The maximum potential AUDPC is simply the AUDPC a variety or clone would have if it had 100% infection at all readings. The maximum potential AUDPC is represented by the dotted line in Figure 1 and is calculated by multiplying the total number of days between the first and last readings by 100.

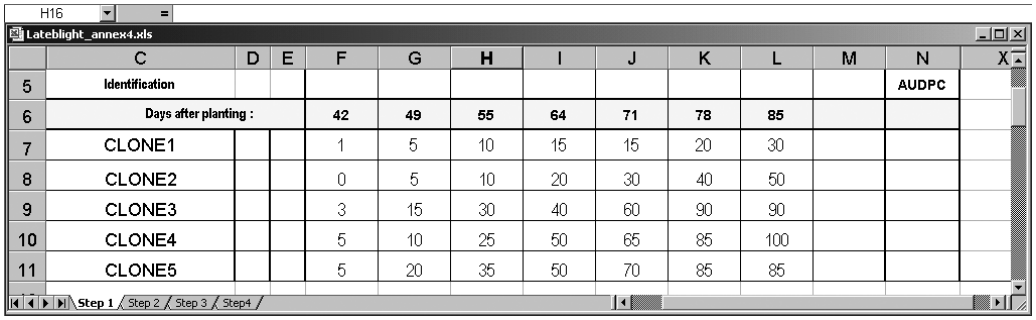
Selection criteria:

AUDPC of the clones is compared to the AUDPC of the susceptible and resistant controls.

Calculating AUDPC with Microsoft Excel

The AUDPC can be calculated using statistical analysis or spreadsheet programs. Here is an example using Microsoft Excel.

Step 1: Enter the evaluation data of the five clones for each of the recording days.



H16 =

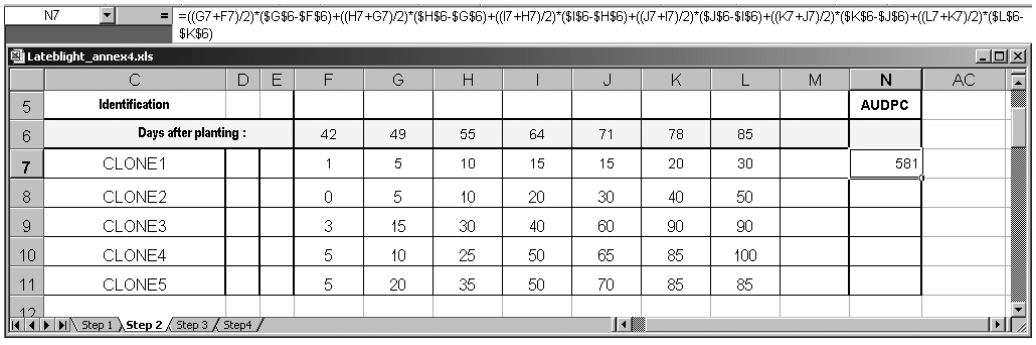
Lateblight_annex4.xls

	C	D	E	F	G	H	I	J	K	L	M	N
5	**Identification**											**AUDPC**
6	**Days after planting :**			**42**	**49**	**55**	**64**	**71**	**78**	**85**		
7	CLONE1			1	5	10	15	15	20	30		
8	CLONE2			0	5	10	20	30	40	50		
9	CLONE3			3	15	30	40	60	90	90		
10	CLONE4			5	10	25	50	65	85	100		
11	CLONE5			5	20	35	50	70	85	85		

Step 1 | Step 2 | Step 3 | Step4

Step 2: Locate the cursor in cell N7, which corresponds to the AUDPC of clone 1, and enter the following formula:

Area = =((G7+F7)/2)*(G6-F6)+((H7+G7)/2)*(H6-G6)+((I7+H7)/2)*(I6-H6)+((J7+I7)/2)*(J6-I6)+((K7+J7)/2)*(K6-J6)+((L7+K7)/2)*(L6-K6)



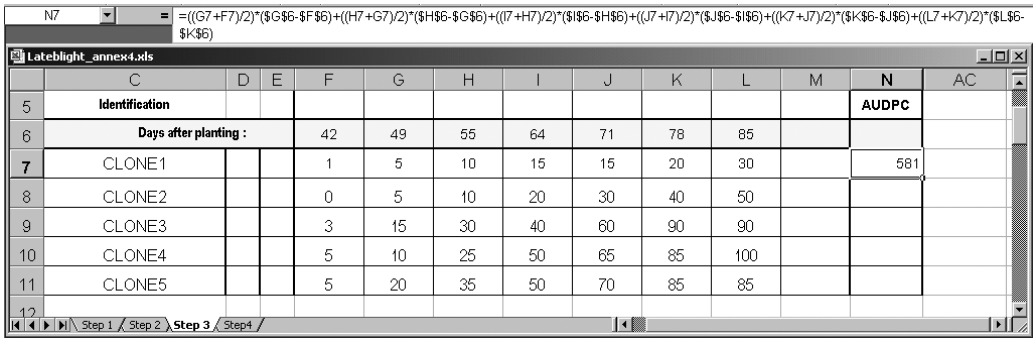
N7 =((G7+F7)/2)*(G6-F6)+((H7+G7)/2)*(H6-G6)+((I7+H7)/2)*(I6-H6)+((J7+I7)/2)*(J6-I6)+((K7+J7)/2)*(K6-J6)+((L7+K7)/2)*(L6-K6)

Lateblight_annex4.xls

	C	D	E	F	G	H	I	J	K	L	M	N	AC
5	**Identification**											**AUDPC**	
6	**Days after planting :**			42	49	55	64	71	78	85			
7	CLONE1			1	5	10	15	15	20	30		581	
8	CLONE2			0	5	10	20	30	40	50			
9	CLONE3			3	15	30	40	60	90	90			
10	CLONE4			5	10	25	50	65	85	100			
11	CLONE5			5	20	35	50	70	85	85			
12													

Step 1 | Step 2 | Step 3 | Step4

Step 3: Press Enter and the area "581" will appear in the cell N7.



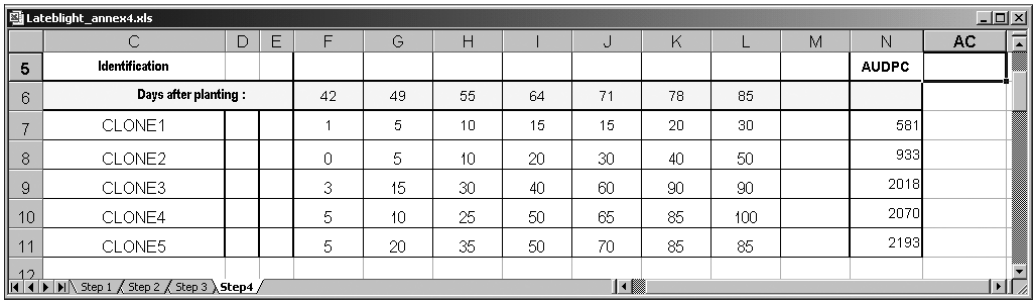
N7 = =((G7+F7)/2)*(G6-F6)+((H7+G7)/2)*(H6-G6)+((I7+H7)/2)*(I6-H6)+((J7+I7)/2)*(J6-I6)+((K7+J7)/2)*(K6-J6)+((L7+K7)/2)*(L6-K6)

Lateblight_annex4.xls

	C	D	E	F	G	H	I	J	K	L	M	N	AC
5	**Identification**											**AUDPC**	
6	**Days after planting :**			42	49	55	64	71	78	85			
7	CLONE1			1	5	10	15	15	20	30		581	
8	CLONE2			0	5	10	20	30	40	50			
9	CLONE3			3	15	30	40	60	90	90			
10	CLONE4			5	10	25	50	65	85	100			
11	CLONE5			5	20	35	50	70	85	85			
12													

Step 1 / Step 2 / Step 3 / Step4

Step 4: Copy the formula of the N7 cell to the other cells from N8 to N11.



Lateblight_annex4.xls

	C	D	E	F	G	H	I	J	K	L	M	N	AC
5	**Identification**											**AUDPC**	
6	**Days after planting :**			42	49	55	64	71	78	85			
7	CLONE1			1	5	10	15	15	20	30		581	
8	CLONE2			0	5	10	20	30	40	50		933	
9	CLONE3			3	15	30	40	60	90	90		2018	
10	CLONE4			5	10	25	50	65	85	100		2070	
11	CLONE5			5	20	35	50	70	85	85		2193	
12													

Step 1 / Step 2 / Step 3 / Step4

Assessing potato clone field resistance to bacterial wilt

Introduction

Ralstonia solanacearum (Smith), causal agent of bacterial wilt

Bacterial wilt (BW) or brown rot is caused by the bacteria *Ralstonia solanacearum* (Smith), previously called *Pseudomonas solanacearum*. The disease is a major constraint to potato production in most tropical and subtropical regions and a threat to the potato seed industry in European countries (EPPO/CABI 1997; Elphinstone 2005). The bacterium affects over 200 species, especially tropical and subtropical crop families, the most susceptible crops being potato, tomato, tobacco, eggplant, pepper, groundnut and banana. In high elevation areas (up to 3,400 masl), potatoes are affected mostly by cool temperature-adapted, restricted host range strains of *R. solanacearum* (race 3/biovar 2A) that are principally transmitted through latently infected tubers.

At lower elevations in the tropics, race 1 strains (biovars 1, 3 or 4) are prevalent and affect a wide range of crops and weeds (Hayward 1991; Priou *et al.* 1999b; Priou *et al.* 2004).

Bacterial wilt symptoms

Above ground symptoms of bacterial wilt include wilting, stunting and yellowing of the foliage. The browning of vascular bundles may be seen when the cortex is peeled. The initial wilting of part of the plant stems, or only one side of the stem or leaf (Picture 1) is also a characteristic symptom. If disease development is rapid, the entire plant wilts quickly, without yellowing (Picture 2). The diseased stem can also wilt completely and dry up, while the remainder of the plant appears healthy (Hayward 1991; Priou *et al.* 2004).

External symptoms on the tuber are visible at harvest when infection is severe. Bacterial ooze collects at tuber eyes causing soil to adhere to the secretions (Picture 3). Tuber symptoms are often described as brown rot. Cut tubers show a pus-like slime coming out of the vascular ring either naturally or when the cut tuber is slightly squeezed. In advanced stages of the infection, tubers exhibit brownish discoloration of the vascular ring (Pictures 4 and 5) (Hayward 1991; Priou *et al.* 2004).

Latently infected tubers do not show visible symptoms but carry the bacteria *Ralstonia solanacearum* and can be responsible for the dissemination of the disease.

Resistance to bacterial wilt

No high level of resistance to bacterial wilt exists in potato cultivars, although some cultivars are less susceptible to BW and can give high yields in the presence of the disease. Healthy seed of a moderately BW-resistant variety can make a huge impact on ware potato production in areas where soils are highly infested (French *et al.* 1998). Cultivars such as Cruza 148 (CIP 720118) do not show wilt in cool conditions, but can disseminate the disease through progeny tubers with a high rate of latent infection.

In the 1990s, advanced potato clones were attained at CIP from a 14-year breeding program for BW resistance. These clones were produced after various crosses with:

- clones derived from Colombian *S. phureja* genotypes produced at the University of Wisconsin in the 1970s. These clones have been found to be strain-specific and sensitive to high temperatures.
- variety AVRDC-1287, derived from *S. chacoense* and *S. raphanifolium*.
- variety Cruza 148 of unknown origin, resistant to BW and late blight.
- diploid populations derived from wild species *S. chacoense, S. sparsipilum and native species S. stenotomum, S. phureja* and *S. goniocalyx*.
- *S. tuberosum subsp. tuberosum* genotypes that carried earliness, adaptation to heat, resistance to late blight and root-knot nematode or both, immunity to potato virus X and Y, high yield and good agronomic characteristics (Schmiediche 1996; French *et al.* 1998).

Breeding for resistance to BW at CIP has resulted in moderate to high levels of resistance to wilt; however, the high frequency of latent infection in tubers is still a problem (Priou *et al.* 2001, 2005). Latent infection is responsible for spread of the disease and overcoming of resistance (French *et al.*, 1998). Because race 3 strain belongs to a genetically homogeneous group, the resistance to race 3 is expected to be more stable than resistance to lowland strains (race 1) of R. solanacearum (French *et al.* 1998). Furthermore, latent tuber infection and above ground plant susceptibility to bacterial wilt are not correlated and the latent clone infection potential does not depend only on wilt incidence but on other factors such as environment (Priou *et al.* 2001).

Picture 1. Plant wilt due to bacterial wilt.

Picture 2. Plant wilt due to bacterial wilt.

Picture 3. Oozing due to bacterial wilt.

Picture 4. Tuber vascular ring due to bacterial wilt.

Picture 5. Tuber damage due to bacterial wilt.

Figure 1. Bacterial Wilt severity scale.

Scale 1. Healthy

Scale 2. ≤ 50% wilted

Scale 3. > 50% wilted

Figure 2. Bacterial Wilt Flow Test.

1. Symptoms identification

2. Stem cutting

3. Sample cleaning

4. Materials

5. Suspension in water

6. Observation

Conducting bacterial wilt intentional exposure evaluation trials

Conditions: climate, soil

Climatic conditions are important for BW development and plant response to the pathogen. In highland tropics, where race 3/biovar 2A strains of R. solanacearum occur more frequently, optimum evaluation conditions are at altitudes ranging from 1,500 to 2,500 masl with average temperatures of 20-22°C. In lower and warmer areas, race 1/biovars 1, 3 and 4 are more likely to occur and BW symptoms will be more severe.

Planting in a loamy to sandy loam soil is preferable.

Materials: clones, control varieties, seed

Clones or varieties from local breeding programs or from CIP can be evaluated.

Local susceptible and moderately resistant varieties are used as controls. The variety Cruza 148 (CIP 720118) is a recommended moderately resistant control; however, special care should be taken with the seed quality because this variety can present high rates of latently infected tubers.

Uniform healthy tubers of the same origin for both advanced clones and controls should be used. A minimum set of 25 tubers/clones is necessary for the first year evaluation. Up to 100 tubers per clone will be necessary to confirm the resistance of the selected clones in the following season.

Inoculum

Clones or varieties can be evaluated in fields naturally infested by R. solanacearum or, if these are not available, on-station fields can be inoculated.

Naturally infested fields. Screening potato for resistance requires a field with a reasonably uniform and moderate level of infestation (between 30 to 50% wilt incidence in the previous potato crop). In the case of heterogeneous soil infestation in the field, the evaluation field is planted with a susceptible potato variety during the previous cropping season. At harvest, rotten tubers are uniformly spread on the field and buried to homogenize and enhance soil inoculum levels.

Field inoculation. One month after potato plant emergence, all the individual plants of the trial, controls included, are inoculated by burying a piece of agar culture that contains approximately 3 x 109 bacteria at the root level (20 cm deep) using a medium size spade. Special care should be taken not to harm the roots of the young potato plants. A 9 cm diameter plate with a 48 h-agar culture of R. solanacearum (local isolate) on modified Kelman's medium without tetrazolium chloride will allow the inoculation of about 8 plants (French *et al.* 1995).

Alternatively, the field can be inoculated before establishing the potato evaluation trial. Three months before planting, tomato plants are transplanted into the fields and are inoculated two weeks later by spraying or dropping a bacterial suspension (3 x 109 bacteria) at the base of the stem. Approximately 6 weeks after inoculation, when at least 80% of plants are wilted, the tomato plants are buried in the soil.

Experimental design

A completely randomized design can be applied in a flat field where the inoculum is randomly distributed.

However, when the field presents a slope, a Randomized Complete Block Design (RCBD) should be used because soil populations of R. solanacearum will not be randomly distributed in the field, but will be displaced with the soil water movement that follows rainfall or furrow irrigation. Inoculum movement can be minimized by building drainage ditches between blocks.

The number of replications used will depend on the uniformity of the distribution of the inoculum within the field.

In a field with uniform distribution of the inoculum, a minimum of 5 replications with 5 plants each should be planted to obtain reliable results. If sufficient amounts of seed tubers are available, the number of plants can be increased up to 20 to 25 plants per experimental unit.

In the case of a heterogeneous distribution of the inoculum within the field, the minimum number of replications is doubled (10) so that the control plots are spread throughout the field and false resistance, due to infection failure resulting from lack of pathogen populations, will be detected.

Field management

Agronomic practices are identical to those recommended for locally grown commercial potato crops. Additionally, the following sanitary precautions should be taken to avoid the spread of the pathogen:

- Workers' shoes and tools are washed and disinfected with 1% sodium hypochlorite when leaving the field.
- Taking into account the field slopes, a 2 meter-deep well is built in the lowest corner of the field to collect run-off water. The well is regularly disinfected with 1% sodium hypochlorite.
- Rotten leftover tubers are removed from the field after harvest.
- Harvested tubers that are not taken to the laboratory for evaluation are burned or used exclusively for food consumption.

Evaluation parameters

Before the growing season

***R. solanacearum* biovars/races identification.** Both races 1 and 3 of R. solanacearum can be found in the field, therefore it is strongly advised to characterize the strains present in naturally infested soils. Pathogens are isolated from soil, diseased plants or tubers, and isolate biovars are identified following the procedures described by French *et al.* (1995).

During the growing season

Plant wilt severity
Plant emergence should be recorded 45 days after planting. The field should be observed regularly starting 45 days after planting to check for the appearance of the first symptoms in the susceptible control varieties. The first evaluation will take place either 60 days after planting, 1 month after inoculation, or as soon as the first symptoms can be observed in the susceptible control. Further observations will be made every 15 days until 2 weeks before harvest.

For each plant, the wilt severity is evaluated using a three-point scale (Figure 1):

1 = healthy plant
2 = below or equal to 50% plant wilted
3 = above 50% plant wilted and eventually dead

The diagnosis of bacterially wilted plants should be confirmed in the field using the vascular flow test. The test consists of cutting a 2-3 cm long piece of stem at the plant base and suspending it in a glass container full of clear water. The cut stem is maintained in a vertical position with the help of an opened paper clip. The exudation of smoke-like milky threads from the cut stem after a few minutes (Figure 2) will confirm the infection by *Ralstonia solanacearum*.

At harvest

Symptomatic tubers

At harvest, the weight of healthy looking tubers and of tubers exhibiting visible BW symptoms (e.g., visible oozing at the eyes or vascular oozing visible upon slicing rotten tubers) is recorded separately. The marketable yield (eventually by market categories) can also be recorded.

The percentage of symptomatic tubers is computed by recording the number of symptomatic tubers and the number of healthy looking tubers in all 5 plants of the experimental unit during the first evaluation year or in 10 plants of the two middle rows of the experimental unit in the following years.

Latently infected tubers

Only asymptomatic tubers of selected clones (see selection criteria below) are analyzed for latent infection by R. solanacearum. For each clone and over all replications, a minimum of 30 tubers of any size above 30 mm and without visible symptoms is selected at random in the field (Priou *et al.* 2001).

Once in the laboratory, sample tubers are washed, disinfected and checked again. At this stage, tubers exhibiting BW symptoms are removed and the number recorded.

Individual asymptomatic tubers are then analyzed with the ELISA test. The CIP kit to perform the post-enrichment enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) is used according to the protocol indicated in the kit manual[1] (Priou *et al.* 1999a).

(1) Please look at CIP BW web page for kit information and order at: http://www.cipotato.org/potato/pests_disease/BacterialWilt/wilt.htm

Recording and analyzing data

Data recording and computation

Average wilt severity score

The average wilt severity score is computed for each experimental unit and evaluation date according to the formula below.

$$\text{Average wilt severity score} = \frac{\sum \text{wilt scores of evaluated plants}}{\text{Total number of evaluated plants}}$$

Average wilt severity scores for each clone/variety can then be computed over the 5 replications.

NB: In case of plant death due to a cause other than bacterial wilt, the plant data should be registered in the data sheet and not included in further computation.

Percent of symptomatic tubers

For each experimental unit, the percent of symptomatic tubers is expressed as a weight, a value which is useful to determine yield losses (Kg/ha.), and as a number of infected tubers, a value which is used for the calculation of infected tuber rates.

$$\text{Percent weight of symptomatic tubers} = \frac{\text{Weight symptomatic tubers}}{\text{Weight healthy looking tubers} + \text{Weight symptomatic tubers}} \times 100$$

$$\text{Percent of symptomatic tubers} = \frac{\text{Number symptomatic tubers}}{\text{Number healthy looking tubers} + \text{Number symptomatic tubers}} \times 100$$

The average percent of symptomatic tubers for each clone/variety is computed over the 5 replications.

Percent of latently infected tubers

For each experimental unit, the percent of latently infected tubers is calculated as follows:

$$\% \text{ Latently infected tubers} = \frac{\text{N° of lab symptomatic tubers.} + \text{N° of NCM- ELISA positive tubers}}{\text{N° of tubers within the sample}} \times 100$$

The average percent of latently infected tubers for each clone/variety is computed over the 5 replications.

Percent of total infected tubers

For each experimental unit the percent of total infected tubers is calculated taking into account the percent of symptomatic tubers found on the experimental unit and the percent of latently infected tubers found in a sample of usually 30 asymptomatic tubers. The percent of total infected tubers is calculated as follows:

$$\% \text{ Total infected tubers} = \frac{\% \text{ symptomatic tubers} + (\% \text{ healthy looking tubers} \times \% \text{ latently infected tubers})}{100}$$

Example: Assuming that the percent of symptomatic tubers is 10, the percent of healthy looking tubers is 90, and from these, 30% infected tubers were detected in the lab, thus the percent of infected tubers will be 10 + (90 x 30/100) = 37%

The average percent of total infected tubers for each clone/variety is computed over the 5 replications.

Data analysis

Last evaluation average wilt scores, percent weight of symptomatic tubers and percent of infected tubers can be analyzed after the exploration of data through simple statistics such as means, standard errors, frequency distributions and boxplots.

Average wilt scores are analyzed using nonparametric statistics and means are compared using Friedman (RCBD) or Kruskall Wallis (RCB) tests.

Variance analysis (ANOVA) is performed with percentage data and means are compared using the Waller Duncan test. Percentage variables do not need to be transformed before the analysis.

Analysis of residuals is recommended to test the validity of the model and analyze the behavior of the variance (homogeneous or not).

These analyses can be performed using R or other statistical packages. CIPSTAT, which uses the R package, facilitates analysis and reports of the results.

Data interpretation

Validation of the experiment

The moderately resistant control should present a wilt score less than or equal to 1.3 and the susceptible control should present a wilt score greater than 1.6 in order to validate the evaluation trial. If both conditions are not observed, the intentional exposure trial will have to be repeated.

Selection criteria

The following selection scale can be used to describe the clones' levels of resistance.

Resistance levels	Average wilt severity score	Percent of total infected tubers (visible + latent)
Highly resistant	1	0
Resistant	1	£15
Moderately resistant	1.01 - 1.3	£30
Moderately susceptible	1.31 - 1.60	Variable
Susceptible	1.61 - 2.2	Variable
Highly susceptible	2.21 - 3.00	Variable

Only clones with an average wilt score less than or equal to 1.3 are selected and will be evaluated again during the next season to confirm their resistance. If Cruza 148 was used as a moderately resistant control variety, clones that show average wilt scores significantly equal or smaller than the control are selected.

Tuber infection rates are highly variable (but unlikely to be null) among moderately to highly susceptible clones because infection of tubers depends not only on wilt severity but also on soil texture, humidity and temperature. However, clones are selected as resistant only if they had no wilt AND a percent of total infected tubers (symptomatic + latent) below or equal to 15%. If the infected tuber rate is higher than 15%, clones will be rated as moderately resistant or even moderately susceptible if the infected tuber rate is higher than 30%.

Assessing potato clone field resistance to leaf miner fly

Introduction

Leaf miner fly, *L. huidobrensis*

Unlike most leaf miner fly species, *Liriomyza huidobrensis* is highly polyphagous and readily develops tolerance or resistance to insecticides. Leaf miner flies have shown significant levels of resistance to most carbamate, organophosphate, and pyrethroid insecticides, commonly used to kill larvae and adult flies. This species originated in the neotropics and was restricted to the Americas until the 1980s. Since then, *L. huidobrensis* has been rapidly spreading to other parts of the world, becoming a key pest in all countries where it has been introduced. The leaf miner fly is capable of complete destruction of a potato field in the absence of control measures (Picture1).

Leaf miner life cycle

The leaf miner fly has four life stages: egg, larvae, pupae and adult. The whole cycle can last 3-5 weeks (Picture 2) and annual reproduction rates can reach up to 13 generations per year. The rounded and translucent eggs are deposited inside the leaf tissue (days 3-6). The larvae period has three stages (days 5-8), which are indicated by the length and thickness of the mine. They can be found by opening the main vein of the leaves. The pupae develop on top of the leaves (days 10-17). After this period, the pupae fall to the ground and remain there until they emerge as fully developed adults. Adults are small, black flies (1.7-2.3 mm long) with a bright yellow spot on the thorax. Mating occurs 6-24 hours after adult emergence and oviposition during the following 1-3 days.

Leaf miner fly damage

Adult damage

Adult females of *L. huidobrensis* use their ovipositor to make holes on the top and bottom sides of the leaves, causing the production of exudates that feed adult males and females. This type of wound is known as the “feeding puncture” (Picture 3). When wounds are made to deposit eggs, they are called “oviposition punctures”.

Larvae damages

The larval stage is the most destructive because the mining reduces the photosynthetic ability of the plant (Picture 4). Larval damage in a growing plant follows a fairly well-established pattern: the larvae attack the plant from the bottom, and later the rest of the plant becomes infested as well. The first leaves to show symptoms are those of the lower part of the potato plant. Middle and upper leaves exhibit progressive damage as the plant grows and infestation continues. Damage in the upper leaves usually occurs when the plant stops growing. Necrosis of the infested leaves follows the same pattern, until the whole plant dries out. Examples of damage levels are shown in Picture 5.

Resistance to leaf miner fly

The rate and level of infestation of plants are affected by varietal differences, age, and the physiological state of the plant.

Seasonal variation of the leaf miner fly population

Climatic conditions are important for the development of the leaf miner fly and can result in clear seasonal trends in population levels. Monthly average temperatures between 13.2 and 18.0ºC support the development of leaf miner fly. These conditions can be found in the Cañete Valley of Peru, where high adult infestation occurs in the winter and spring months of June to October while moderate adult infestation occurs in the warmer months of December to April.

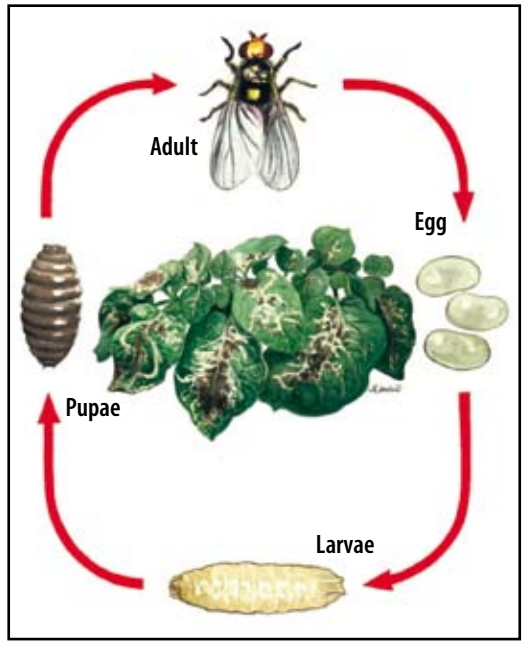
Conducting leaf miner fly intentional exposure evaluation trials

Conditions: screening site

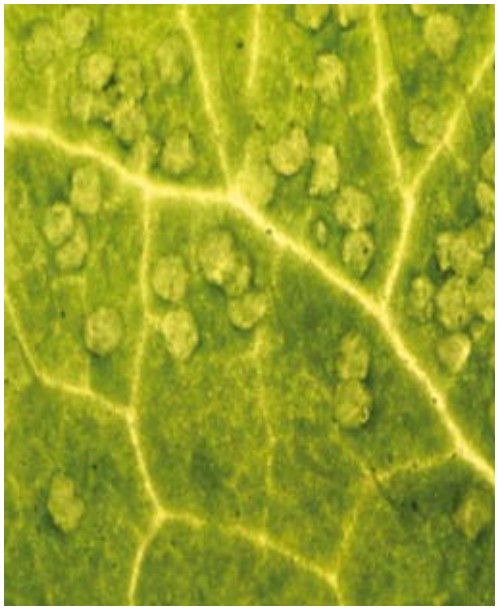
The procedure for evaluation of leaf miner fly resistance of advanced potato clones involves conducting trials over two seasons in an area of high incidence of the fly. The cultivation of host plants (such as beans, fava beans and snow peas) in the area surrounding the trial helps to build high population levels of leaf miner fly.

In the case of seasonal variation in population levels, the first evaluation should be done under moderate adult infestation. After elimination of the most susceptible

Picture 1. Destruction of a potato plant.



Picture 2. Life cycle of the leaf miner fly.



Picture 3. Feeding punctures produced by female adults.

Picture 4. Larvae damage.

Picture 5. Damage levels on the variety Revolution.

60 days after planting

75 days after planting

90 days after planting

clones, the remaining selected clones are re-evaluated under high adult infestation pressure in the second season to confirm resistance.

Materials: clones, control varieties, seed

Twenty tubers of each clone should be available to begin the evaluation trials. A minimum of 90 tubers should be used during the second season, which also allows for yield evaluation of the potato clones.

All evaluation trials must include a susceptible local variety as a control. The varieties Canchan and Desiree are often used as susceptible controls. However, in the case of evaluation under high infestation, a resistant variety such as Maria Tambeña should be included as well. It is important to include controls of the same maturity as the tested material.

Because of high temperatures during the growing season, tubers should be renewed for each evaluation.

Experimental design

Clones are separated into groups of 15 to 25. Experimental design is a randomized complete block design (RCBD) with 3 replications when the trial also involves yield evaluation or when blocking is necessary because of the close presence of host plants that will affect the distribution of leaf miner flies within the trial. When none of those conditions are fulfilled, a complete random design (CRD) should be used.

During the first evaluation, experimental plots consist of one row with 10 plants. For the following evaluations, experimental plot size will increase up to 3 rows with 10 plants/row under moderate adult infestation and up to 5 rows with 5 to 10 plants/row under high adult infestation.

Managing the field trial

Agronomic practice in the experimental area will be similar to that of a local commercial potato field. Pesticide applications can be carried out against late blight and mites as needed, but other pesticides should not be applied.

Monitoring population levels

The objective of monitoring the leaf miner fly population is to study its population dynamics. Adults are monitored by using one yellow sticky trap (20cm x 20 cm) placed above the canopy in the middle of the experimental field. The traps are changed weekly from 30 until 90 days after planting. The height of the trap is adjusted to the plant growth each time a new trap is installed. The number of leaf miner flies stuck to the trap is registered in the section 7 of the Pest monitoring data sheet of the ICG field book.

Evaluating foliage damage

Three readings of cumulative percent of foliage damage will be made randomly on 5 individual plants within each experimental unit. Border plants or rows are excluded from sampling. Readings are taken at 60, 75 and 90 days after planting for early clones and 75, 90, and 105 days after planting for late maturing clones.

Recording and analyzing data

Data recording

For each sample plant, damage can be recorded on a scale from 1-5 or as a percent of leaf area affected by leaf miner fly. The scale is described in the Assessment for Resistance to Leaf Miner datasheet (LeafMinerFly worksheet) of the ICG field book in which all data from the experiment are registered.

Data analysis

Statistical analysis is performed only when susceptible controls show more than 75% of leaf damage.

Percent of leaf damage can be analyzed using variance analysis (ANOVA) after the exploration of data through simple statistics such as means, standard errors, frequency distributions and box plots.

Percent of leaf damage is a pseudo-quantitative variable with hierarchy. Such data can be analyzed without transformation using a nonparametric test such as the

Friedman (RCBD) or the Kruskall Wallis (RCB) tests. Other tests can be used depending on the data distribution.

These analyses can be performed using R or other statistical packages. CIPSTAT, which uses the R package, gives analysis and reports on the results.

Data interpretation

Validation of the experiment

The evaluation of resistance to leaf miner fly is considered valid if the susceptible control presents a score damage over 3 (>50% plant damage) under moderate adult leaf miner fly infestation, and score damage over 4 (>70% plant damage) under high adult leaf miner fly infestation.

Selection criteria

Under valid conditions, resistance of the tested clones can be evaluated by comparison to the controls. Evaluated material can be separated into highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS). The criteria presented in Table 1 can also be used after verifying that the controls belong to their appropriate category.

Table 1. Evaluation scale to screen for leaf miner fly resistance in potato clones.

Foliage damage (%)	Level of resistance	Score
0	Highly resistant (HR)	1
1-25	Resistant (R)	2
26-50	Moderately resistant (MR)	3
51-75	Susceptible (S)	4
>75	Highly Susceptible (HS)	5

Assessing potato clone resistance to virus diseases

Introduction

Potato virus Y (PVY) and Potato leaf roll virus (PLRV) are the two most important virus diseases of potato, responsible for serious yield losses worldwide. Potato virus X (PVX), although less important alone, is commonly found in infections combined with other viruses, increasing the disease effect.

This protocol describes intentional exposure trials for assessment of resistance to these three viruses in advanced selection programs. A standardized methodology to assess the presence of extreme resistance to PVX and PVY is also described.

Pathogen /Vector

Potato viruses are small transmissible pathogens which use components of host plant cells to multiply. PVX is easily transmitted by contact through knives; secateurs; or movement of equipment, people and animals across a field.

PLRV is transmitted by several potato-colonizing aphid species, but *Myzus persicae* is regarded as the most efficient vector. Both larval and adult stages of winged and nonwinged forms transmit PLRVs. Aphid transmission of PLRV is described as "persistent". PLRV is acquired and transmitted over long periods. A latency or incubation period is needed between acquisition and inoculation of the virus, which can persist throughout the aphid's life. The aphid vectors of this virus can be controlled by application of aphicides, although resistance to pesticides is common.

As with PLRV, PVY is also transmitted by aphids, including potato-colonizing and noncolonizing species and, again, *Myzus persicae* is regarded as the most efficient vector (including larval and adult stages of winged and nonwinged forms). However, more than 30 species of noncolonizing aphids can also act as vectors for PVY. Aphid transmission of PVY is nonpersistent. Acquisition and inoculation of PVY requires only seconds, but aphids rapidly lose their virus charge after brief feeding on healthy plants and must again feed on a PVY-infected plant to transmit PVY virus. It is generally not possible to control the spread of PVY by using aphicides.

The three viruses are also vegetatively spread through tubers or stem cuttings but are not known to be transmitted through botanical ("true") seed.

Symptoms

In the process of infecting potatoes, viruses often damage or disrupt a plant's normal growing pattern, causing such visible symptoms as leaf mottle, leaf distortion, and plant stunting. Slower plant growth, inefficient use of nutrients and reduced tolerance to other stresses can also be caused by virus infection. In potatoes, leaf mosaics reduce the leaf area available to produce food.

PVX symptoms include mild leaf mottle or yellow mosaic between veins (Picture 1).

Plants infected by PVY will present symptoms of leaf bunching and crinkling, mild or strong leaf mosaic, necrotic veins, leaf spots and leaf dropping (Picture 2).

Symptoms of PLRV include blockage of the food movement channels of the plant. Upper leaves in primary infected plants[1] become pale, upright and rolled, showing some reddening of the tissue around the leaf edges. The lower leaves may or may not have symptoms. Secondary infected plants[2] develop severely rolled lower leaves (Picture 3) which feel like leather. The plant frequently has an overall stunted, upright, chlorotic appearance. The oldest leaves may show reddening on the margins or chlorosis (Picture 4). The upper leaves may not have obvious symptoms.

Resistance to PVX and PVY

Until now, three different types of resistance to PVX and PVY have been recognized: extreme resistance, hypersensitivity, and relative resistance (Fernandez-Northcote 1991).

Extreme resistance refers to the highest level of resistance and is the most stable with respect to pathogen variants and environmental conditions. Clones with extreme resistance (ER) inhibit virus multiplication to such an extent that no virus can be recovered from plants by indexing[3]. Extreme resistances to PVX and PVY are each controlled by single dominant genes. This is the type of resistance utilized in CIP's virus resistance breeding program.

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(1) Primary symptoms result when an initially healthy plant becomes infected mechanically or by aphids during the current season.

(2) Secondary infection occurs when an infected tuber is planted, giving rise to an infected plant.

(3) "Indexing" refers to testing for viral infection by serological methods or transfer to indicator plants by either mechanical, vector or graft inoculation.

Picture 1. Strong symptoms of leaf mosaic and crinkling.

Picture 2. Systemic necrosis of veins and stems.

Picture 3. Rolled lower leaves and chlorotic appearance.

Picture 4. Stunting and reddening of the margins.

Hypersensitivity (HR) is a variant-specific resistance, characterized by a local necrotic reaction at the initial infection site which contains the virus and hampers its spread throughout the plant. High temperatures can modulate expression of HR, reducing its efficiency and resulting in the development of multiple larger necrotic lesions in leaves and other parts of the plant (systemic necrosis).

In the case of PVX, the local necrosis (tiny necrotic spots on the inoculated leaf) observed after mechanical inoculation is a desirable hypersensitivity reaction governed by the N genes for virus resistance (Fernandez-Northcote 1991). However the hypersensitive "top necrosis" (typical necrosis of apical shoots) reaction that can be observed after graft inoculation is considered an undesirable hypersensitivity reaction and is classified as extreme susceptibility.

The hypersensitive reaction to PVY is more difficult to observe and is apparently due to the interaction of minor modifier genes with the Ny genes for hypersensitivity. (Fernandez-Northcote 1991).
Relative resistance, which is polygenic in nature, confers varying degrees of resistance to virus infection.

Resistance to PLRV

Relative resistance is the main and most common type of resistance encountered against PLRVs. Usually, sources of PLRV resistance present a polygenic inheritance, influenced by environmental factors such as vector and inoculum pressure. Two components of relative resistance to PLRV have been identified: resistance to infection and resistance to multiplication. The former is the most studied and most frequently used in breeding programs. This type of resistance results in low rates/frequencies of infected plants following exposure to PLRVs. Infection rates depend on the level of susceptibility of the clone and the vector pressure encountered during the growing season.

Conducting trials for evaluating relative resistance to PVX and PVY

Conditions: screening site

Evaluation field trials for relative resistance to PVX and PVY require the testing material to be exposed to virus infection during two previous seasons in a location and season of high aphid population and virus incidence.

The evaluation *per se* takes place during the third season under identical conditions of exposure to virus.

Materials: clones, control varieties, seed

Seeds are multiplied under conditions of high disease severity during 2 consecutive seasons. Twenty tubers of each clone should be available to begin the seed multiplication during the first season. At harvest, three random tubers are collected from every plant of each plot and carried forward to the implementation of the next multiplication field. The process will be repeated during the second season, increasing both the number of seeds and the level of infection. Tuber seeds harvested during the second season will be used for the third season intentional exposure trial.

Control varieties are used to verify the efficiency of the inoculum; therefore, tuber seed of a local susceptible cultivar is included in all trials. CIP uses the varieties Perricholi, Tomasa, Tito and Condemayta. While the European varieties Atlantic and Bintje are susceptible to PVY and Granola is susceptible to PVX, CIP does not use these varieties because they are not well-adapted to the tropics.

Additional inoculum is usually not required, as PVX and PVY can easily be mechanically transmitted by human activities such as rubbing of plant stems and leaves, spraying, and cultivating and damaging tubers in bins or harvesters. PVY can also be transmitted by unexpected visiting aphids.

Infector plants can also be used to spread the viruses. Infectors are plants grown from the PVY and PVX infected tubers of a susceptible local cultivar. One infector plant tuber can be planted at one end of every plot.

Experimental design

During the two first seasons, the material is planted in replicated plots randomly distributed. A minimum of two replications of ten hill-plots is used during the first season while the number of replications is increased in the following seasons.

The evaluation trials take place in the third season and are conducted in Randomly Complete Block Design (RCBD) with a minimum of three replications of ten hill-plots.

Yield reduction due to the effect of virus infection can also be estimated during the third exposure trial by planting, for comparison purpose, a trial identical to the intentional exposure trial [same entries (including controls), same design, and same location] using healthy tuber seed.

Field Management

Field management is carried out according to local procedures. Plants are spaced at 0.30m intervals on the row. During the three seasons, roguing of infected plants is avoided because the presence of infected plants is necessary for the evaluation of relative resistance in subsequent growing cycles.

Monitoring the aphid population

The first aphid count occurs 40 days after planting on a random sample of 50 plants. Since aphids tend to be located on the underside of the leaves, each leaf should be turned up carefully to allow an accurate counting of winged and wingless aphids. Two additional counts can be performed at 20 day intervals. The data are recorded in the section 8 of the Pest monitoring data sheet of the ICG field book. The average number of aphids per plant is computed. These data will provide information on vector population density for PVY transmission.

Levels of aphid population can be evaluated by placing yellow tray traps containing water (40x30x6cm) in the field (Fernandez-Northcote 1991). Counts are performed with the same frequency mentioned above. At each counting, the trays are removed and new traps are placed in the field.

Evaluation parameters to assess PVX and PVY infection

During the first two seasons, infection is confirmed through the careful observation of all experimental plots. In the first season, observations of symptoms are conducted as late as 60 or 70 days after planting because susceptible plants will acquire infection for the first time (primary infection) late during the growing season. Due to the presence of secondary infection, observations are carried out earlier during the second season. PVX- and PVY-infected clones may be identified by careful inspection of hill-plots, early in the morning or under overcast skies for better visualization of symptoms. ELISA serology tests can be conducted on susceptible controls to trace the level of infection.

In the evaluation trial, observations of symptoms are conducted 45-50 days after planting for early maturing clones and 60-70 days for later ones. The ELISA test is performed on all of the plants in each plot to detect asymptomatic infected plants and to confirm infection by a given virus.

To conduct the ELISA serology test, a sample of two or three asymptomatic leaflets and two or three symptomatic leaflets is taken, put in a small polyethylene plastic bag and sent to the laboratory. A positive result in ELISA confirms infection of the plant with the respective indexed virus.

Yield data are taken into account in the third season and include the total number of plants harvested, total number of tubers and total weight for each plot. Those data are collected in the intentional exposure trial and in the healthy seed trial if available.

Recording and analyzing data

Data recording

In each plot the total number of plants, the number of plants showing mosaic and/or systemic necrosis symptoms and the number of plants positive in the ELISA test to a specific indexed virus are recorded in the Assessment for Virus Resistance datasheet (virus worksheet) of the ICG field book.

The percentage of infected plants is computed for each experimental unit.

Yield data of the intentional exposure trial are registered in the same datasheet.

Data analyzing

Percent of infected plants can be analyzed using variance analysis (ANOVA) after the exploration of data through simple statistics such as means, standard errors, frequency distributions and boxplots.

To analyze the effect of virus-infected seed on yield, a combined analysis of variance of the two trials (healthy seed tuber trial vs virus infected tuber trial) is performed if there is homogeneity in the error variance of the two experiments. The error mean square of the combined ANOVA is used to carry out statistical comparison between yields of each clone in the two trials. Paired test comparisons are used to compare each entry.

Results can be presented using the following table:

Identification	% infected plants	% yield loss	LSD	significance

Data interpretation

Validation of the experiment

Susceptible controls should reach more than 80% of infection in the third exposure trial to obtain reliable results on the levels of relative resistance.

Selection criteria

Advanced clones with 25% or fewer plants infected with PVX and/or PVY in the third exposure trial are considered relatively resistant to the respective virus.

The clones that do not suffer yield reduction despite high rates of virus infection are considered tolerant. Clones that have not shown any infected plants with PVY and/or PVX may undergo laboratory graft and mechanical inoculation to determine presence of extreme resistance.

Conducting evaluation for extreme resistance (ER) to PVX and PVY

Conditions: climate, soil

This trial is conducted under greenhouse conditions during seasons when maximum temperatures oscillate between 19 to 24 °C and minimum between 15 and 21 °C. This trial takes place during the fall and spring in the lowland tropics of Peru. Separate greenhouses are used to test each virus.

Materials

The following material is necessary to conduct the tests:

A. A set of seven plants per clone and per virus to be tested. Plants can be obtained from tubers, cuttings or sprouts
B. A set of seven plants of a PVX-susceptible, PVY-susceptible and extremely resistant potato cultivar to be used as controls. Costañera is a Peruvian variety with extreme resistance to PVX and PVY.
C. A set of young PVX-infected plants of *Nicotiana glutinosa* for mechanical inoculation: 40 infected plants are enough to mechanically inoculate 100 plants.
D. A set of PVX-infected plants of a susceptible cultivar that develops evident mosaic symptoms against PVX for graft inoculation. The cultivar Rosita is used at CIP. Cultivars that develop necrosis are not desirable since they transmit the virus less successfully. One plant is sufficient to graft inoculate three or four plants. In each plant, the number of apexes (future scions) is multiplied by cutting the principal apex or plant tip 30 - 40 days after planting or when the plants are 40 cm tall.
E. A set of young PVY-infected plants of *Nicotiana occidentalis* for mechanical inoculation: 40 infected plants are enough to mechanically inoculate 100 plants.
F. A set of young PVY-infected plants of *Nicotiana occidentalis* for graft inoculation: one plant is sufficient to graft inoculate three or four plants if the number of apexes (future scions) is multiplied by cutting the principal apex or plant tip 30-40 days after planting or when the plants are 40 cm tall.
G. A set of healthy young plants of *Nicotiana glutinosa* and *Nicotiana occidentalis* for indexing. Three plants per test clone are necessary.
H. ELISA kit

Inoculum

Inoculations are carried out using the PVX strain member of Cockerham's group 2 of PVX strains and the PVY^{O} strain of PVY which induces severe mosaic and necrosis.

Picture 5a. Nicotiana glutinosa plant infected with PVX.

Picture 5b. Nicotiana occidentalis plant infected with PVY.

Picture 6. Preparing inoculum for mechanical inoculation.

Picture 7. Mechanical inoculation of test clones.

Picture 8. Graft inoculation of test clones (PVY-infected scion of N. occidentalis).

Picture 9. Grinding leaflets for indexing.

These strains are maintained in plants of N. glutinosa and N. occidentalis respectively by mechanical inoculation (Pictures 5a and 5b).

Inoculum is prepared at 5% w/v which means that 5g of symptomatic leaves (N. glutinous for PVX and N. occidentalis for PVY) are needed for each 100 ml of cold water. Leaves are ground in a mortar with cold distilled water and the suspension is then strained using a piece of cheesecloth to remove the debris (Picture 6).

Experimental design

No design is used in this evaluation. The only restriction in the organization of the material is that controls are grown separately while inoculated (mechanical/graft) plants are grouped by entry.

Management of the experiment

Mechanical inoculation

Three plants of each set of seven plants are mechanically inoculated with PVX and PVY, respectively, 15 days after planting, *i.e.*, when plants are still young or about 10 cm in height. Two or three leaves of the test clones are slightly dusted with 600 mesh carborundum and inoculated by rubbing the leaflets with the forefinger (use gloves) previously dipped in the inoculum (Picture 7).

Graft inoculation

Three additional plants in each set are graft inoculated 25 days after planting, or when plants are 25 to 30 cm in height. Inoculation is carried out by grafting scions of infected plants onto the individual potato plants to be tested (Picture 8). Scions are obtained from infected plants of a PVX susceptible potato cultivar and PVY-infected plants of *N. occidentalis*. Scions are removed from plants 20 days after grafting to avoid any passive translocation of the virus that could be detected by ELISA.

Control

The remaining (healthy control) plants of each set are kept separately in the same greenhouse.

Sampling for ELISA test

ELISA is performed on composite samples of leaves of plants inoculated with the same virus and inoculation technique, *i.e.*, a sample of leaves from 3 plants mechanically inoculated with the same virus. Clones that develop systemic necrosis usually escape virus detection by ELISA, however detection is possible when a 2 cm apical section of the main stem is included into the sample.

Back testing on indicator plants

Clones that show negative results in ELISA are indexed on virus-free indicator plants of *N. glutinosa* for PVX and *N. occidentalis* for PVY detection.

Preparation of the inoculum. A mixed sample of leaflets taken from all plants of the putative resistant clone is crushed in cold distilled water (1:5) inside a small plastic bag. For the clones that develop systemic necrotic symptoms, a piece of the apical part of the stem should be added to the sample. A glass test tube (25 cm^3) is used to crush and grind the leaflets by rolling and rubbing the tube over the plastic bag, maintaining the open side of the plastic bag folded to avoid loosing the suspension (Picture 9).

Inoculation: A set of two young plants of each virus indicator is dusted with 600 mesh carborundum, and inoculated by gently rubbing a few leaves with forefingers previously dipped in the inoculum (Picture 7). The indicator plants are labeled with the code or name of the tested clone.

Evaluation parameters: reading and assessment

Evaluations are recommended for the plants 25 days after mechanical or graft inoculation, although the evaluation can start as soon as symptoms appear on the susceptible variety.

Plants of each clone are individually assessed for mosaic (M), systemic necrosis (SN), and local necrotic symptoms (LN).

In the back testing, evaluation is carried out on the indicator plants 15 days after inoculation. The plants are assessed for mosaic symptoms. An ELISA test can be carried out to confirm the presence or absence of virus.

Recording and analyzing data

Data are recorded in the Assessment for Extreme Resistance to Virus datasheet (PVX inoculate or PVY inoculate worksheet) of the ICG field book.

Data interpretation

Any doubtful symptoms should be compared with the respective healthy control.

If the tested clones do not show any symptoms, if the ELISA test is negative and if none of the indicator plants shows mosaic symptoms, the tested clone can be declared extremely resistant.

Conducting trials for evaluating relative resistance to PLRV

Conditions: climate, soil, aphid pressure

Assessment of relative resistance to PLRV infection requires three seasons of intentional field exposure to PLRV infection. The location and season should be selected for high PLRV incidence and aphid pressure as the resistance to PLRV infection depends on aphid pressure.

The evaluation per se takes place during the third season. An area with moderate aphid pressure is recommended when the resistance of the test material is unknown but some level of resistance is suspected, while an area with high aphid pressure is recommended when a high level of resistance is known to be present in the material.

Materials: clones, control varieties, seed

Test clones

Twenty tubers of each clone should be available during the first season and at least 30 tubers should be available during the following seasons.

Controls

Healthy tuber seed of a local susceptible cultivar should be included as a susceptible control. In CIP, the varieties Perricholi and Ticahuasi are susceptible controls. Tomasa, Tito, Condemayta, Achirana-INTA and the Polish-bred line DW.84-1457 are used as resistant controls.

Seed

Over the seasons, the harvested tubers from test clones, controls and infector plants are carried forward as seed for the next trial. At harvest, three random tubers are collected from every plant of each plot.

Infectors

Infector plants should be present in each infection exposure field as a source of inoculum to spread the virus. Infectors are obtained from tubers of PLRV-infected

plants of a susceptible cultivar grown under natural field infection for at least two seasons (to assure infection) and in the same location where the intentional exposure trials will be carried out. However, once infected, PLRV infector tubers can also be propagated vegetatively without exposure to aphid pressure. At CIP, infector seed are obtained by multiplying two susceptible local cultivars, Perricholi and Ticahuasi, in an area with high PLRV incidence.

Vectors

In locations where aphid populations might be low, aphid multiplication can be planned in advance. Aphids of the *Myzus persicae* species are multiplied on Chinese cabbage (*Brassica perkinensis*) one month before the trials are set. The aphid propagation takes place in an isolated greenhouse with artificial light, with temperatures ranging between 19 and 24°C and relative humidity ranging between 65 and 80%.

Experimental design

Over the two first seasons, replicated plots of materials to be tested are planted under a randomly replicated design with two replications of ten hill-plots for the first season, and three replications of ten hill-plots for the next. During the third season, the evaluation trials are conducted in RCBD with at least three replications of ten hill- plots.

Two or three tubers of infector plants should be planted at one end of every plot (figure 1). Infectors are planted two weeks before the test material in order to allow the early acquisition of virus by the aphids.

Yield reduction due to the effect of virus infection can also be estimated during the intentional exposure trial by planting, for comparison, a trial identical to the intentional exposure trial (same entries, same design, and same location) using healthy tuber seed.

Field Management

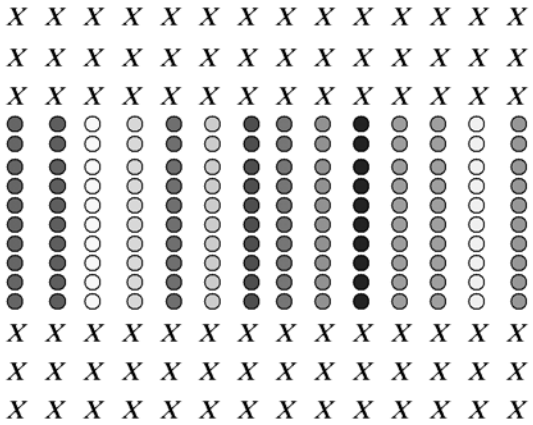
Field management is done according to local procedures.

Monitoring aphid population levels

Aphid counting should be performed regularly in each trial in order to determine the level of aphid pressure (Table1).

Figure1. Field design for a replication of the exposure trial.

(*X* = infector plants; round circles = plants of test clones)



The first counting occurs 40 days after infectors are planted. A random sample of 50 infector plants is taken. Since aphids tend to be located on the underside part of the leaves, each leaf should be turned up carefully and winged and wingless aphids should be counted. Two or three additional countings can be performed at 20 day-intervals. The average number of aphids per plant is computed on each occasion.

Aphid population level can also be evaluated by placing yellow tray traps containing water (40x30x6cm) in the field (Fernandez-Northcote 1991).

Table 1. Aphid pressure (*Myzus persicae*).

Aphid pressure	Number of aphids/ trap/ month	Number of aphids/plant
High	≥ 500	≥ 50
Moderate	250	20-49
Low		≤ 20

Decreasing the aphid population

If the mean number of aphids/plant is equal to or greater than 50, the application of a contact pesticide (*i.e.*, Pirimor 0.2%) is recommended to moderate the aphid pressure.

Increasing aphid population

If the mean number of aphids/plant is less than or equal to 10, the aphid population needs to be increased. Full leaves of 'Chinese cabbage' heavily infested with aphids (*Myzus persicae*) will then be set over the infector plants.

Evaluation Parameters

PLRV symptoms during the first exposure season can be observed 65 or 70 days after planting. It is important to know that plants acquiring the virus for the first time (primary infection) do not show conspicuous symptoms unless they become infected very early in the season, *i.e.*, during the first thirty days after planting.

During the second season, symptoms on the secondary infected plants are more noticeable.

Evaluation during field exposure trials (third season) are performed on individual plants at 45 or 50 days after planting.

ELISA is conducted on leaf samples of both symptomatic and asymptomatic plants.

Yield data are taken into account in the third season and include the total number of plants harvested, total number of tubers and total weight for each plot. The same measures should be taken in the healthy seed trial planted the same season for comparison analysis.

Recording and analyzing data

Data recording

The numbers of symptomatic and ELISA-positive infected plants are registered into the Assessment for Virus Resistance datasheet (virus worksheet) of the ICG field book for each clone and replication. The percent of infected plants is computed for each experimental unit. This should be based on ELISA-positive plants or on symptomatic plants if ELISA was not performed.

Yield data of the intentional exposure trial are registered in the same datasheet.

Data analyzing

Percentage of PLRV-infected plants can be analyzed using variance analysis (ANOVA) after the exploration of data through simple statistics such as means, standard errors, distribution frequencies and boxplots.

In order to analyze the effect of virus-infected seed on yield, a combined analysis of variance of the two trials (healthy seed tuber trial vs virus infected tuber trial) is performed if there is homogeneity in the error variance of the two experiments. The error mean square of the combined ANOVA is used to carry out statistical comparison between yields of each clone in the two trials. Paired test comparisons are used to compare each entry.

Results can be presented using the following table:

Identification	% PLRV infected plants	% yield loss	LSD	significance

Data interpretation

Validation of the experiment

Susceptible controls should achieve more than 80% of infected plants in the third exposure trial to obtain reliable results on the levels of relative resistance.

Selection criteria

Clones that exhibit 50% or more infected plants (symptomatic plants) during the second season are declared susceptible to PLRV infection and are not carried forward to the intentional exposure trial.

Clones with 30% or fewer PLRV infected plants (positive to ELISA) during the intentional exposure trial (3rd season) may be considered resistant to PLRV infection.

The clones that do not suffer yield reduction despite high rates of virus infection are considered tolerant.

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Glossary

AMMI analysis
Additive Main effect and Multiplicative Interaction analysis

Aphid multiplication
Aphids of the Myzus persicae species should be reared in plants of Brassica perkinensis or Datura stramonium species before an experiment for virus resistance evaluation is set. Aphids are propagated in an isolated nethouse with light intensity over 5000 lux, a range of temperature between 19 to 24˚C and relative humidity between 65 and 80%.

AUDPC
Area Under the Disease Progress Curve

BIBD
Balanced Incomplete Block Design

CRD
Completely Randomized Design

GGE biplot
Graphic method for analyzing multi-environment trials

Indexation
Indexation refers to testing for viral infection by serological methods or transfer to indicator plants using either mechanical, vector or graft inoculation

Indexing by serology
Use of the ELISA (Enzyme Linked Immunosorbant Assay) or similar protein-based test for the detection of specific plant viruses

LAI
Leaf Area Infection

MET
Multi-Environment Trials

MLVT
Multi-Locational Variety Trials

RCBD
Randomized Complete Block Design

Roguing
Roguing is the manual removal of plants from the field. Note that above and below ground parts of virus-infected plants should be destroyed upon removal from the field to avoid re-emergence of infected plants, or the return of viruliferous vectors to infest the remaining healthy plants.

SET
Standard Evaluation Trial

Annex 1

A brief description of commonly used incomplete block designs

Balanced Incomplete Block Design (BIBD)

Characteristics

Each block in a balanced incomplete block design does not contain all treatments; the precision of comparisons between treatments differs depending if the treatments belong to the same block or not. The design is called "balanced" when the experiment is planned in such a way that every pair of treatments occurs the same number of times. Balanced incomplete block design can be used when assessing potato clone processing and cooking performance using a panel of evaluators in which each evaluator tastes/characterizes a limited number of clones. Each evaluator represents a block and evaluates a different selection of potato clones/varieties.

Design

Organizing an experiment with a balanced incomplete block design requires use of the following parameters:

- t = number of treatments (ex. 15 clones)
- b = number of blocks (ex. 15 tasters)
- k=number of experimental units within each block having k<t (ex. 10: each taster can evaluate 10 clones)
- r = number of times each treatment appears having r < b
- λ= number of blocks in which the i^{th} treatment and the j^{th} treatment appear together (λ is the same for all pairs of treatments)

The parameters have to be chosen carefully so that $bk = tr$ and $\lambda (t\text{-}1) = r (k\text{-}1)$. All parameters should be whole numbers. Cochran and Cox (1957) provide tables assigning suitable values to parameters.

For example, if a taster is able to taste up to 8 clones ($k = 8$), the tasting of 15 potato clones ($t = 15$) by 15 tasters ($b = 15$) can be arranged in a balanced incomplete block design. The design will provide 8 repetitions ($r = 8$) for each clone and the same paired combination of clones will tasted by 4 different tasters ($\lambda = 4$).

Randomizing

Since the design is linked to many constraints, it is appropriate to use design generated by software, or pre-established tables (Cochran and Cox, 1957). Score sheets with appropriate sample selections that respect the design can be prepared in advance as shown below:

FRENCH FRY SCORE SHEET

Test Number:_____ Panelist _____Juez 1_____ Date:_____

Factor	Sample number									
	1	3	7	8	10	11	12	13		
External appearance										
Excellent										

FRENCH FRY SCORE SHEET

Test Number:_____ Panelist _____Juez 2_____ Date:_____

Factor	Sample number									
	2	5	6	7	8	10	14	15		
External appearance										
Excellent										

FRENCH FRY SCORE SHEET

Test Number:_____ Panelist _____Juez 3_____ Date:_____

Factor	Sample number									
	2	4	7	8	9	12	13	15		
External appearance										
Excellent										

Split-Plot Design

Characteristics

The split-plot design is a special kind of incomplete block design. The underlying principle of the split-plot design is that whole plots, subject to one or more treatments (factor A), are divided into subplots to which one or more additional treatments are applied (factor B). Thus, each whole plot may be considered as a block for subplot treatments (factor B), but only as an incomplete block as far as the full set of treatments is concerned (factor A + B). The design may be used when an additional factor (such as planting density or fertilizer use) is to be incorporated into an experiment to increase its scope.

Randomization

Randomization is a two-stage process. First, factor A treatments are randomized over the whole plot; then factor B treatments are randomized within the subplots. Randomization at each stage can be done with a table of random numbers or by using MS Excel.

A3	B2	2 1 3 4 5
	B1	3 5 4 1 2
	B3	4 1 5 3 2

A1	B3	4 3 5 2 1
	B2	1 5 4 3 2
	B1	3 5 1 4 2

A2	B2	3 2 1 5 4
	B3	3 2 4 5 1
	B1	5 1 4 2 3

Partially Balanced Lattice Design

Characteristics

The partially balanced lattice design is recommended when the number of treatments is very large or when the experimental units are very heterogeneous. Lattice designs are incomplete block designs. Each block does not contain all treatments, so the precision of comparison between treatments differs depending if the treatments belong to the same block or not. The lattice design (also called double lattice or square lattice), is a partially balanced design in which the number of treatments is a perfect square (9, 16, 25, 36, 49, 64, 81, 121 etc.) and the number of treatments within each block is equal to the square-root of the total number of treatments. This design needs two or multiples of two replications. The experimental units within each incomplete block should be as homogeneous as possible.

Randomizing

Treatments are arranged in the form of a square (step 1). Treatments are grouped by row, and then by columns. The row grouping is generally known as X grouping. The group of treatments in one row will form a block. All the rows (blocks) will make one repetition (step 2). The column grouping is generally known as Y grouping. The

group of treatments in one column will constitute another block. This Y grouping will form the other repetition (step 3). The X grouping and Y grouping ensure that treatments occurring together in the same block once do not appear together in the same block again.

For each repetition, the randomization is a three-stage process: the blocks are randomized, each treatment is randomized within each block (step 4), and ultimately a treatment is randomly assigned to each plot.

Step 1: Arrangement of treatments into a square

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

Step 2: repetition 1 (grouping by row or X-grouping)

Block 1	1	2	3	4	5
Block 2	6	7	8	9	10
Block 3	11	12	13	14	15
Block 4	16	17	18	19	20
Block 5	21	22	23	24	25

Step 3: repetition 2 (grouping by column or Y-grouping)

Block 6	1	6	11	16	21
Block 7	2	7	12	17	22
Block 8	3	8	13	18	23
Block 9	4	9	14	19	24

Step 4: randomization

1- repetition
2- block
3- treatment

Block 8	3	18	23	13	8
Block 10	10	5	25	20	15
Block 9	14	4	9	24	19
Block 6	16	11	21	1	6
Block 7	22	17	7	2	12
Block 5	22	23	25	21	24
Block 1	4	2	1	3	5
Block 3	12	15	14	11	13
Block 2	6	8	10	9	7

Annex 2

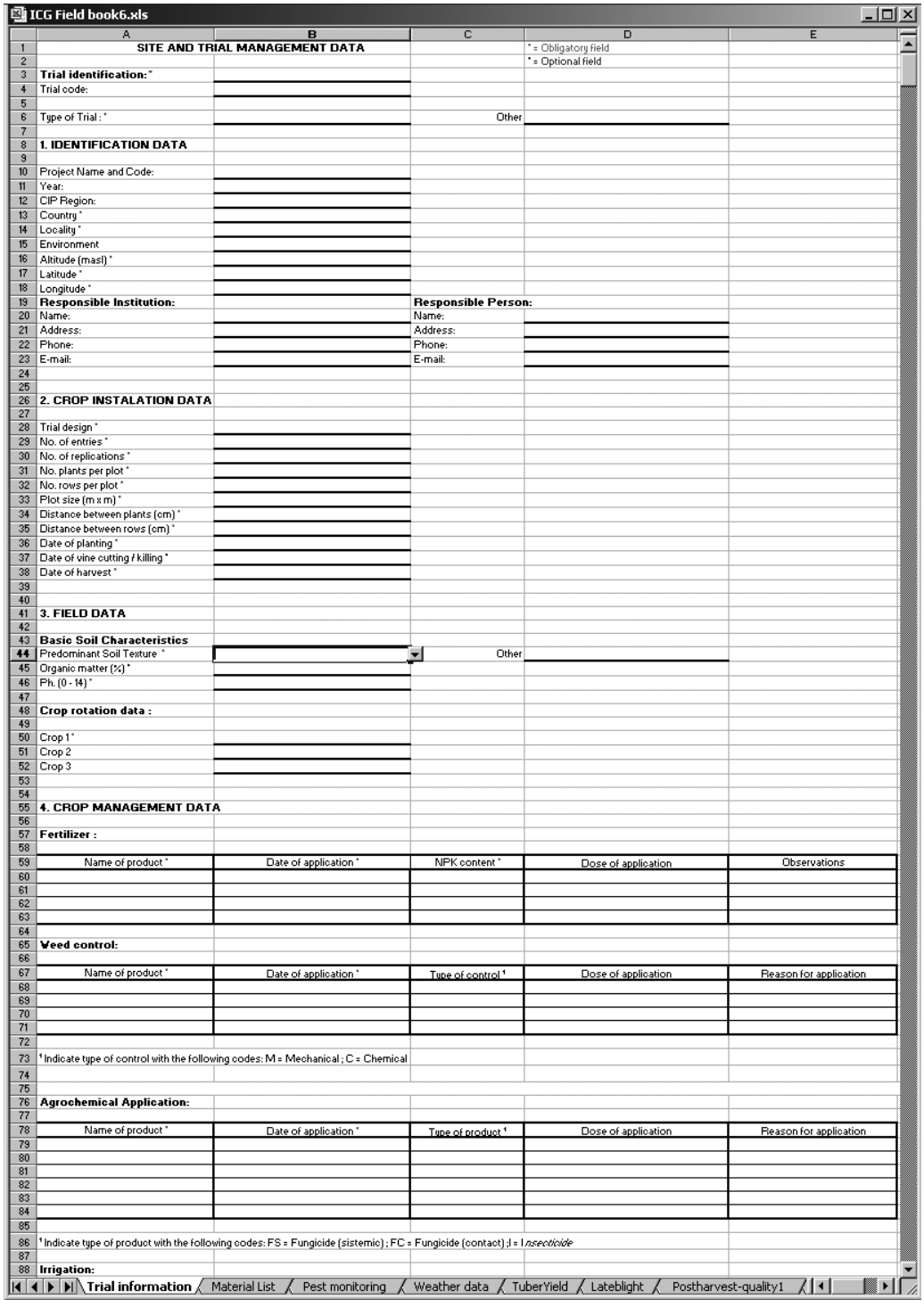
Field book

This document makes reference to a number of worksheets (developed with Excel) to record information and data from the field. The individual datasheets are listed below.

A. Trial information worksheet: Trial identification, site, characterization and management data
B. Material list worksheet: Plant materials list
C. Pest monitoring worksheet: monitoring pests/diseases, leaf miner fly, aphids.
D. Climate data worksheet: field weather data and temperature/humidity in storage area
E. Tuber yield worksheet: Assessment for tuber yield
F. Post-harvest quality 1 worksheet: Assessment for post-harvest quality: dry matter and specific gravity
G. Post-harvest quality 2 worksheet: Assessment for post-harvest quality: Chipping, French-frying, and cooking
H. Post-harvest quality 3 worksheet: Assessment for storage ability
I. Late blight worksheet: Assessment for late blight resistance (intentional exposure)
J. Resistance BW (5 plants) worksheet: Assessment for bacterial wilt (intentional exposure): 5 plants
K. Resistance BW (10 plants) worksheet: Assessment for bacterial wilt (intentional exposure): 10 plants
L. BW Harvest worksheet: Assessment for bacterial wilt (intentional exposure): harvest
M. Leaf Miner Fly worksheet: Assessment for resistance to leaf miner fly (intentional exposure)
N. Virus worksheet: Assessment for virus resistance (intentional exposure)
O. PVX inoculate worksheet: Assessment for PVX virus resistance (greenhouse)
P. PVY inoculate worksheet: Assessment for PVY virus resistance (greenhouse)

Screen captures of the individual worksheets are provided on the following pages as a visual reference.

A. Trial information worksheet: Trial identification, site, characterization and management data

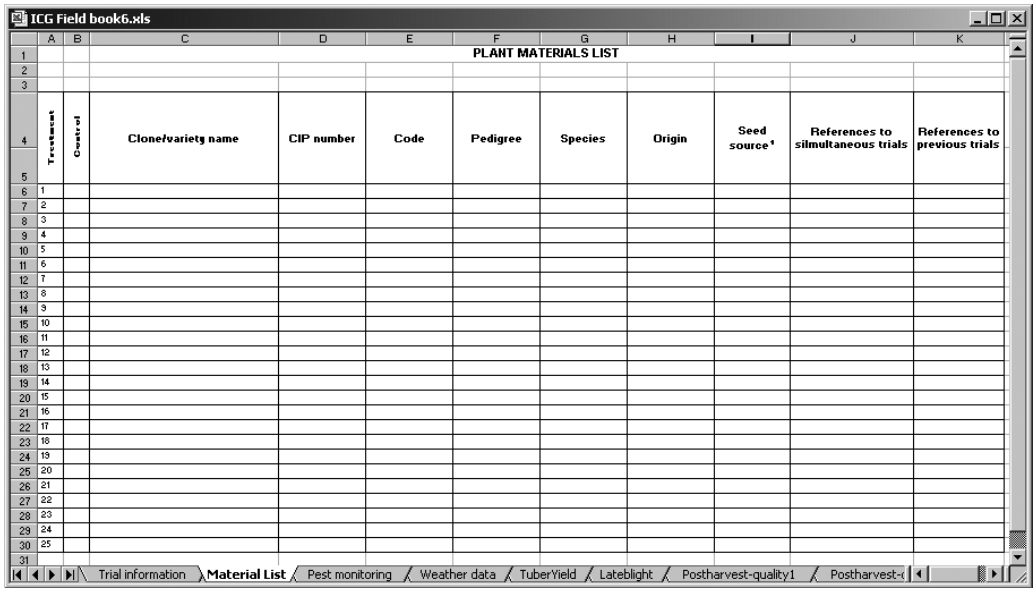


ICG Field book6.xls

	A	B	C	D	E
1	**SITE AND TRIAL MANAGEMENT DATA**			* = Obligatory field	
2				* = Optional field	
3	**Trial identification:** *				
4	Trial code:				
5					
6	Type of Trial : *		Other		
7					
8	**1. IDENTIFICATION DATA**				
9					
10	Project Name and Code:				
11	Year:				
12	CIP Region:				
13	Country *				
14	Locality *				
15	Environment				
16	Altitude (masl) *				
17	Latitude *				
18	Longitude *				
19	**Responsible Institution:**		**Responsible Person:**		
20	Name:		Name:		
21	Address:		Address:		
22	Phone:		Phone:		
23	E-mail:		E-mail:		
24					
25					
26	**2. CROP INSTALATION DATA**				
27					
28	Trial design *				
29	No. of entries *				
30	No. of replications *				
31	No. plants per plot *				
32	No. rows per plot *				
33	Plot size (m x m) *				
34	Distance between plants (cm) *				
35	Distance between rows (cm) *				
36	Date of planting *				
37	Date of vine cutting / killing *				
38	Date of harvest *				
39					
40					
41	**3. FIELD DATA**				
42					
43	**Basic Soil Characteristics**				
44	Predominant Soil Texture *		Other		
45	Organic matter (%) *				
46	Ph. (0 - 14) *				
47					
48	**Crop rotation data :**				
49					
50	Crop 1 *				
51	Crop 2				
52	Crop 3				
53					
54					
55	**4. CROP MANAGEMENT DATA**				
56					
57	**Fertilizer :**				
58					
59	Name of product *	Date of application *	NPK content *	Dose of application	Observations
60					
61					
62					
63					
64					
65	**Weed control:**				
66					
67	Name of product *	Date of application *	Type of control ¹	Dose of application	Reason for application
68					
69					
70					
71					
72					
73	¹ Indicate type of control with the following codes: M = Mechanical ; C = Chemical				
74					
75					
76	**Agrochemical Application:**				
77					
78	Name of product *	Date of application *	Type of product ¹	Dose of application	Reason for application
79					
80					
81					
82					
83					
84					
85					
86	¹ Indicate type of product with the following codes: FS = Fungicide (sistemic) ; FC = Fungicide (contact) ; I = *Insecticide*				
87					
88	**Irrigation:**				

Trial information | Material List | Pest monitoring | Weather data | TuberYield | Lateblight | Postharvest-quality1

B. Material list worksheet: Plant materials list



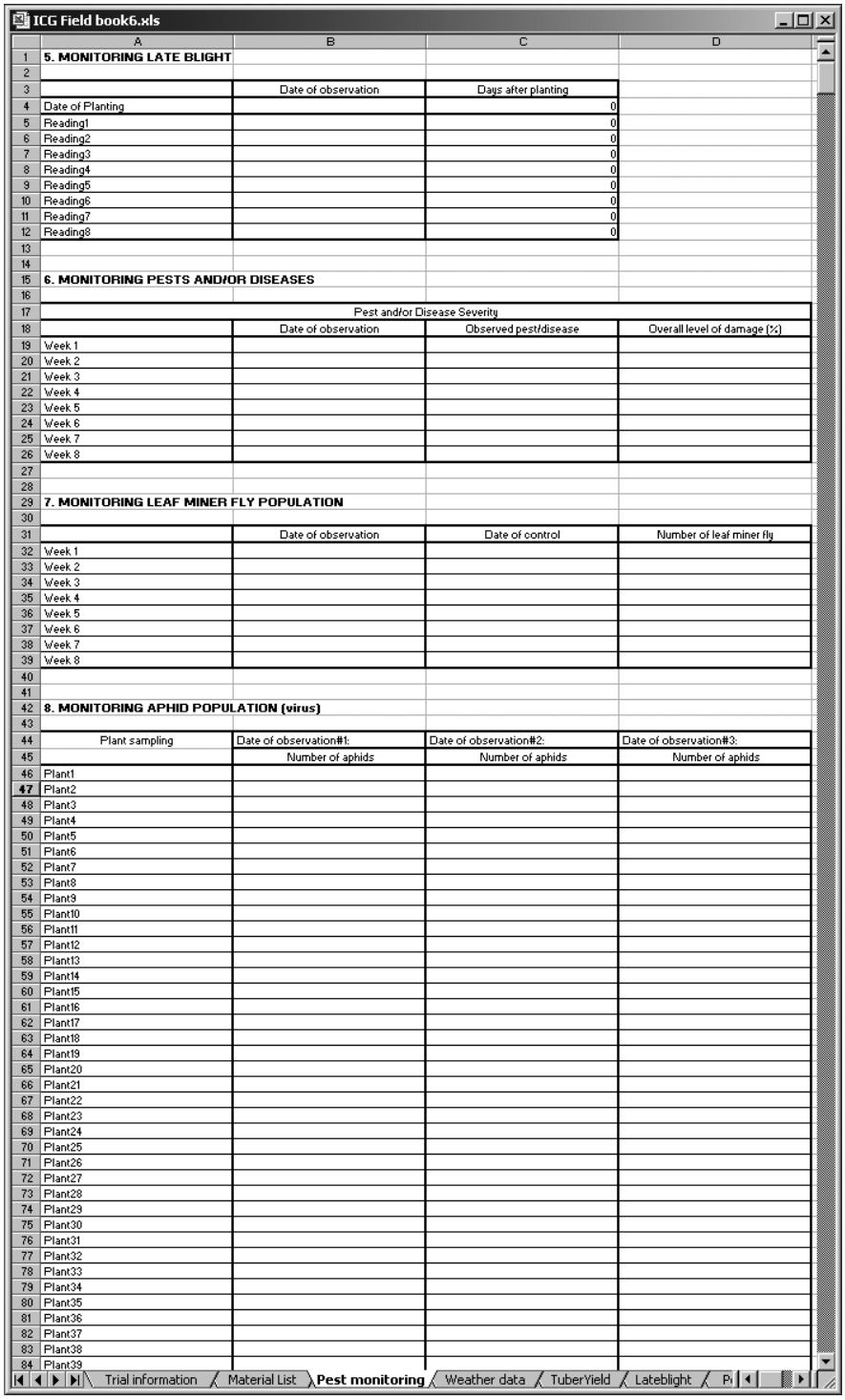
ICG Field book6.xls

PLANT MATERIALS LIST

Treatment	Control	Clone/variety name	CIP number	Code	Pedigree	Species	Origin	Seed source[1]	References to simultaneous trials	References to previous trials
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										

Trial information | Material List | Pest monitoring | Weather data | TuberYield | Lateblight | Postharvest-quality1 | Postharvest-

C. Pest monitoring worksheet: monitoring pests/diseases, leaf miner fly, and aphids.

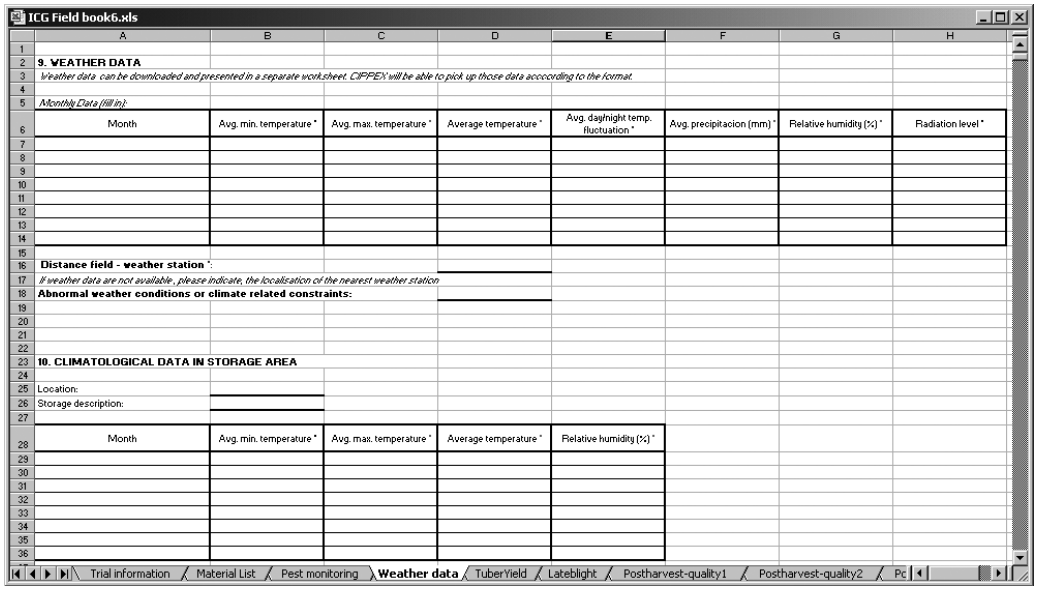


ICG Field book6.xls

	A	B	C	D
1	**5. MONITORING LATE BLIGHT**			
2				
3		Date of observation	Days after planting	
4	Date of Planting		0	
5	Reading1		0	
6	Reading2		0	
7	Reading3		0	
8	Reading4		0	
9	Reading5		0	
10	Reading6		0	
11	Reading7		0	
12	Reading8		0	
13				
14				
15	**6. MONITORING PESTS AND/OR DISEASES**			
16				
17		Pest and/or Disease Severity		
18		Date of observation	Observed pest/disease	Overall level of damage (%)
19	Week 1			
20	Week 2			
21	Week 3			
22	Week 4			
23	Week 5			
24	Week 6			
25	Week 7			
26	Week 8			
27				
28				
29	**7. MONITORING LEAF MINER FLY POPULATION**			
30				
31		Date of observation	Date of control	Number of leaf miner fly
32	Week 1			
33	Week 2			
34	Week 3			
35	Week 4			
36	Week 5			
37	Week 6			
38	Week 7			
39	Week 8			
40				
41				
42	**8. MONITORING APHID POPULATION (virus)**			
43				
44	Plant sampling	Date of observation#1:	Date of observation#2:	Date of observation#3:
45		Number of aphids	Number of aphids	Number of aphids
46	Plant1			
47	Plant2			
48	Plant3			
49	Plant4			
50	Plant5			
51	Plant6			
52	Plant7			
53	Plant8			
54	Plant9			
55	Plant10			
56	Plant11			
57	Plant12			
58	Plant13			
59	Plant14			
60	Plant15			
61	Plant16			
62	Plant17			
63	Plant18			
64	Plant19			
65	Plant20			
66	Plant21			
67	Plant22			
68	Plant23			
69	Plant24			
70	Plant25			
71	Plant26			
72	Plant27			
73	Plant28			
74	Plant29			
75	Plant30			
76	Plant31			
77	Plant32			
78	Plant33			
79	Plant34			
80	Plant35			
81	Plant36			
82	Plant37			
83	Plant38			
84	Plant39			

Trial information | Material List | **Pest monitoring** | Weather data | TuberYield | Lateblight | Pl

D. Climate data worksheet: field weather data and temperature/humidity in storage area

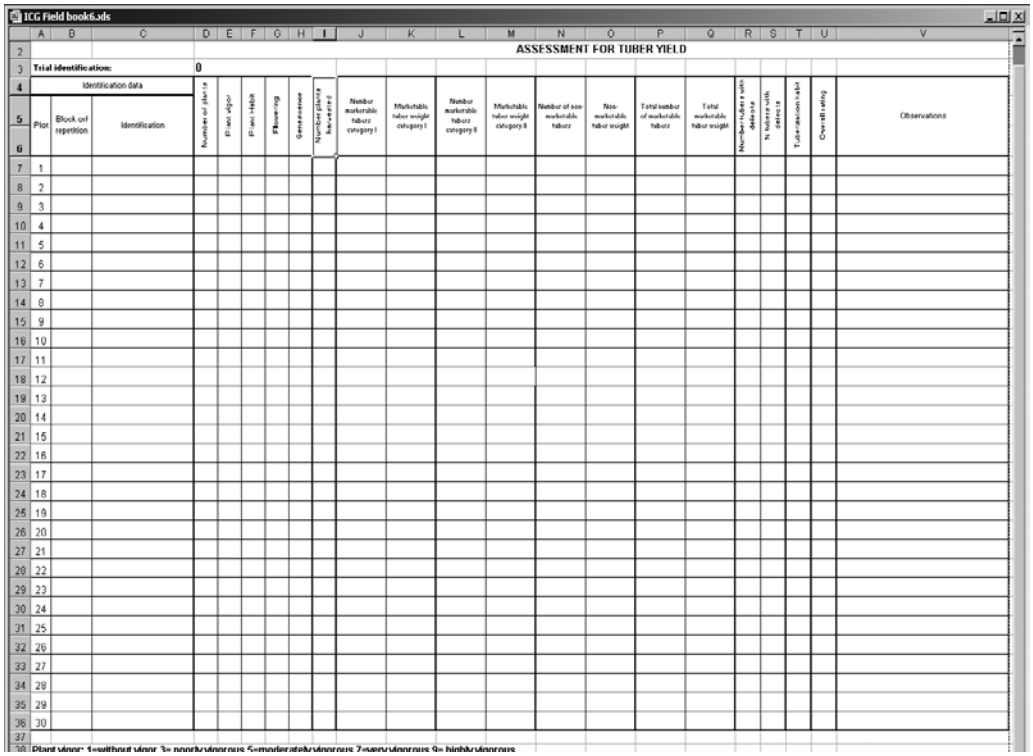


ICG Field book6.xls

	A	B	C	D	E	F	G	H
1								
2	**9. WEATHER DATA**							
3	*Weather data can be downloaded and presented in a separate worksheet. CIPPEX will be able to pick up those data accocrding to the format.*							
4								
5	*Monthly Data (fill in):*							
6	Month	Avg. min. temperature °	Avg. max. temperature °	Average temperature °	Avg. day/night temp. fluctuation °	Avg. precipitacion (mm) °	Relative humidity (%) °	Radiation level °
7								
8								
9								
10								
11								
12								
13								
14								
15								
16	**Distance field - weather station °:**							
17	*If weather data are not available , please indicate, the localization of the nearest weather station*							
18	**Abnormal weather conditions or climate related constraints:**							
19								
20								
21								
22								
23	**10. CLIMATOLOGICAL DATA IN STORAGE AREA**							
24								
25	Location:							
26	Storage description:							
27								
28	Month	Avg. min. temperature °	Avg. max. temperature °	Average temperature °	Relative humidity (%) °			
29								
30								
31								
32								
33								
34								
35								
36								

Trial information | Material List | Pest monitoring | **Weather data** | TuberYield | Lateblight | Postharvest-quality1 | Postharvest-quality2 | Pc

E. Tuber yield worksheet: Assessment for tuber yield



ICG Field book6.xls

ASSESSMENT FOR TUBER YIELD

Trial identification: 0

Identification data			Number of plants	Plant vigor	Plant habit	Flowering	Senescence	Number plants harvested	Number marketable tubers category I	Marketable tuber weight category I	Number marketable tubers category II	Marketable tuber weight category II	Number of non-marketable tubers	Non-marketable tuber weight	Total number of marketable tubers	Total marketable tuber weight	Number tubers with defects	N. tubers with defects	Tuber necrosis habit	Overall rating	Observations
Plot	Block or repetition	Identification																			
1																					
2																					
3																					
4																					
5																					
6																					
7																					
8																					
9																					
10																					
11																					
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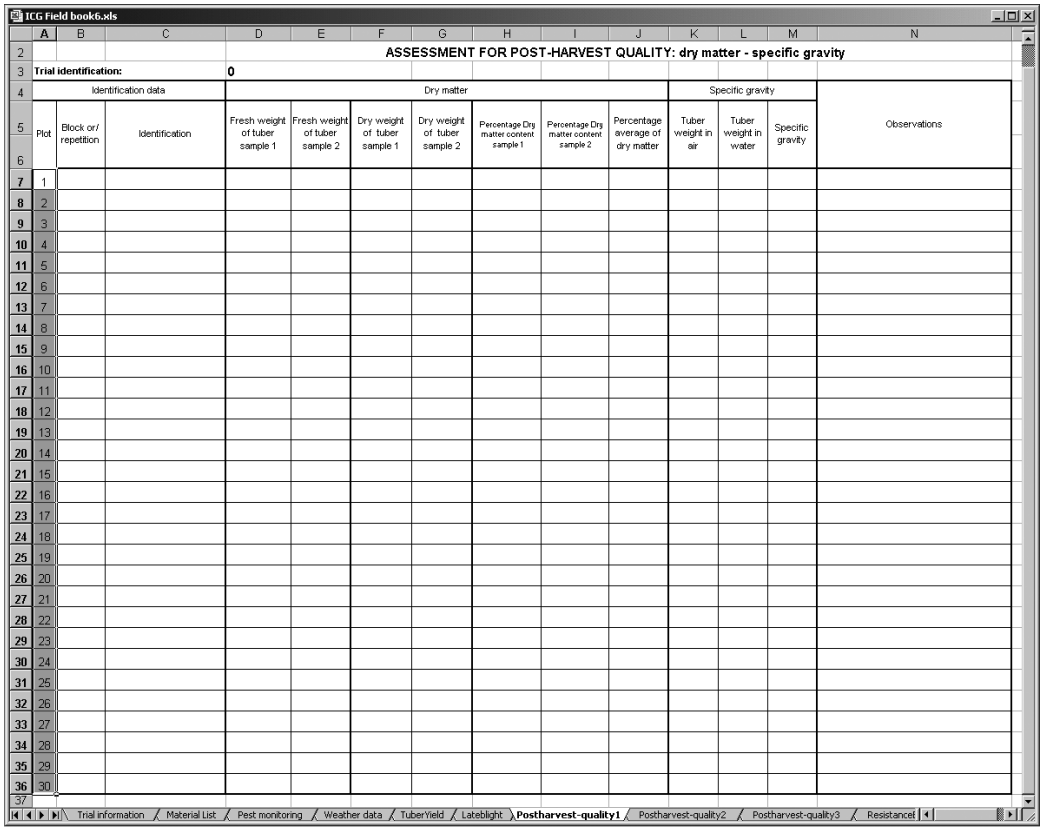
26																					
27																					
28																					
29																					
30																					

Plant vigor: 1=without vigor 3= poorly vigorous 5=moderately vigorous 7=very vigorous 9= highly vigorous

F. Post-harvest quality 1 worksheet: Assessment for post-harvest quality: dry matter and specific gravity.



ICG Field book6.xls

ASSESSMENT FOR POST-HARVEST QUALITY: dry matter - specific gravity

Trial identification: 0

Identification data			Dry matter							Specific gravity			
Plot	Block or/ repetition	Identification	Fresh weight of tuber sample 1	Fresh weight of tuber sample 2	Dry weight of tuber sample 1	Dry weight of tuber sample 2	Percentage Dry matter content sample 1	Percentage Dry matter content sample 2	Percentage average of dry matter	Tuber weight in air	Tuber weight in water	Specific gravity	Observations
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
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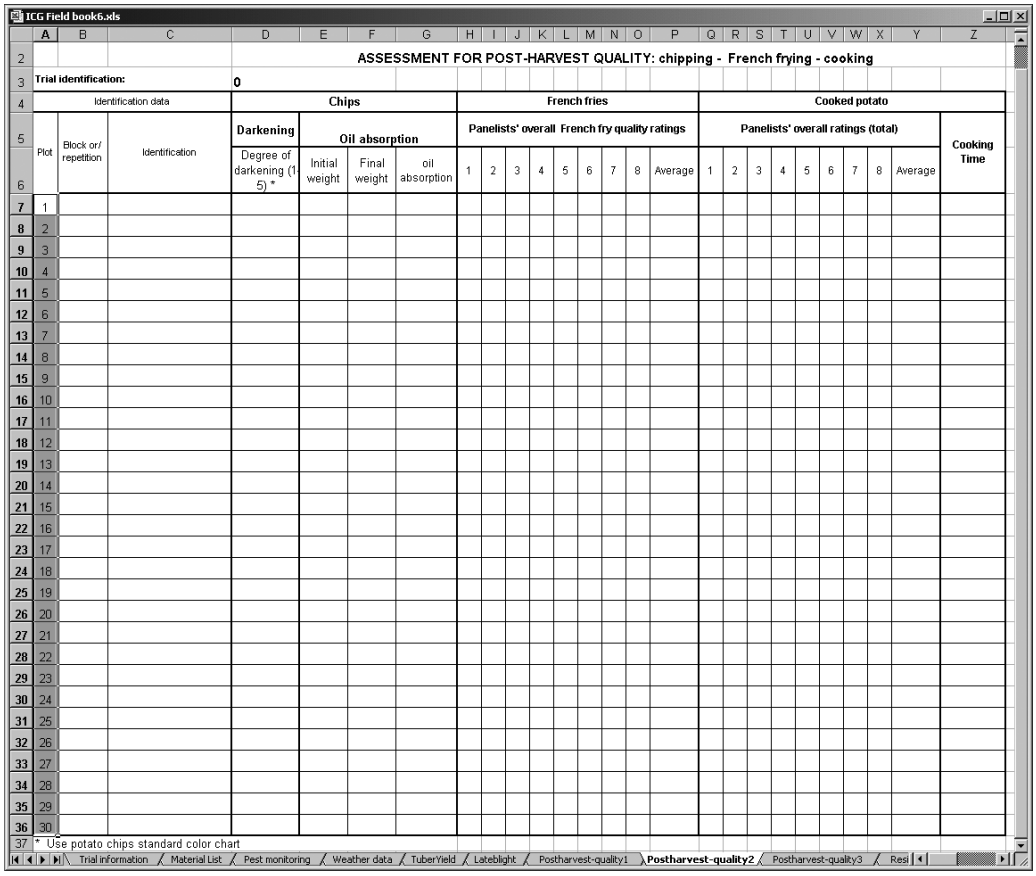
31													
32													
33													
34													
35													
36													

Trial information | Material List | Pest monitoring | Weather data | TuberYield | Lateblight | Postharvest-quality1 | Postharvest-quality2 | Postharvest-quality3 | Resistance

G. Post-harvest quality 2 worksheet: Assessment for post-harvest quality: Chipping, French-frying, and cooking



ICG Field book6.xls

ASSESSMENT FOR POST-HARVEST QUALITY: chipping - French frying - cooking

Trial identification: 0

Identification data			Chips				French fries									Cooked potato									
			Darkening	Oil absorption			Panelists' overall French fry quality ratings									Panelists' overall ratings (total)									Cooking Time
Plot	Block or/ repetition	Identification	Degree of darkening (1 5) *	Initial weight	Final weight	oil absorption	1	2	3	4	5	6	7	8	Average	1	2	3	4	5	6	7	8	Average	
1																									
2																									
3																									
4																									
5																									
6																									
7																									
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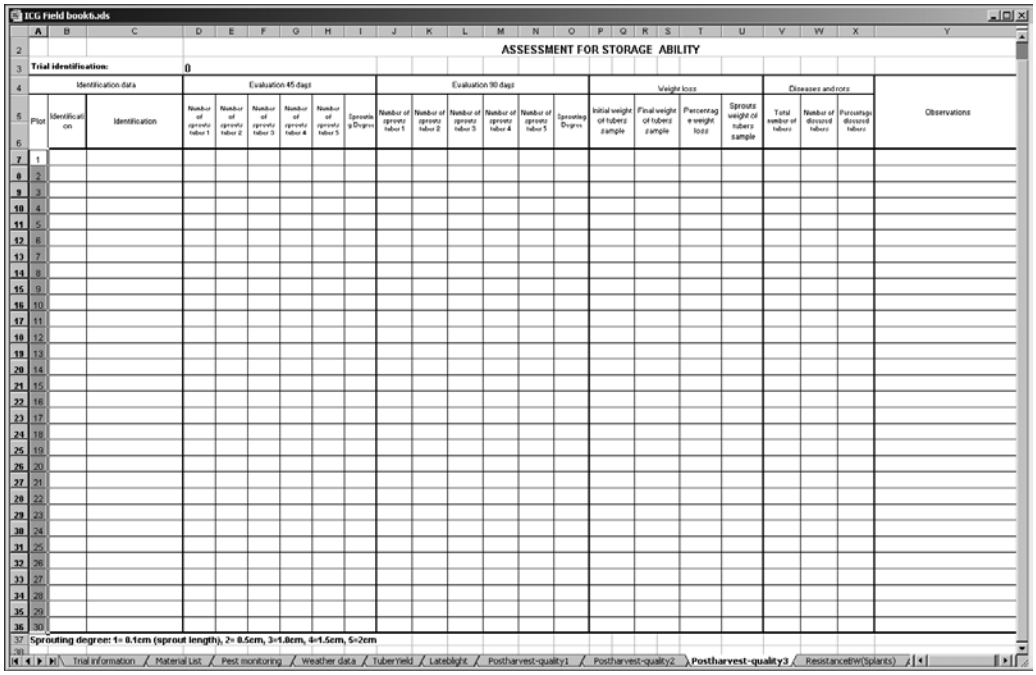
28																									
29																									
30																									

* Use potato chips standard color chart

Trial information | Material List | Pest monitoring | Weather data | TuberYield | Lateblight | Postharvest-quality1 | **Postharvest-quality2** | Postharvest-quality3 | Resi

H. Post-harvest quality 3 worksheet: Assessment for storage ability



ICG Field book6.xls

ASSESSMENT FOR STORAGE ABILITY

Trial identification: 0

Identification data			Evaluation 45 days						Evaluation 90 days						Weight loss				Diseases and rots			
Plot	Identification	Identification	Number of sprouts tuber 1	Number of sprouts tuber 2	Number of sprouts tuber 3	Number of sprouts tuber 4	Number of sprouts tuber 5	Sprouting Degree	Number of sprouts tuber 1	Number of sprouts tuber 2	Number of sprouts tuber 3	Number of sprouts tuber 4	Number of sprouts tuber 5	Sprouting Degree	Initial weight of tubers sample	Final weight of tubers sample	Percentage weight loss	Sprouts weight of tubers sample	Total number of tubers	Number of diseased tubers	Percentage diseased tubers	Observations
1																						
2																						
3																						
4																						
5																						
6																						
7																						
8																						
9																						
10																						
11																						
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23																						
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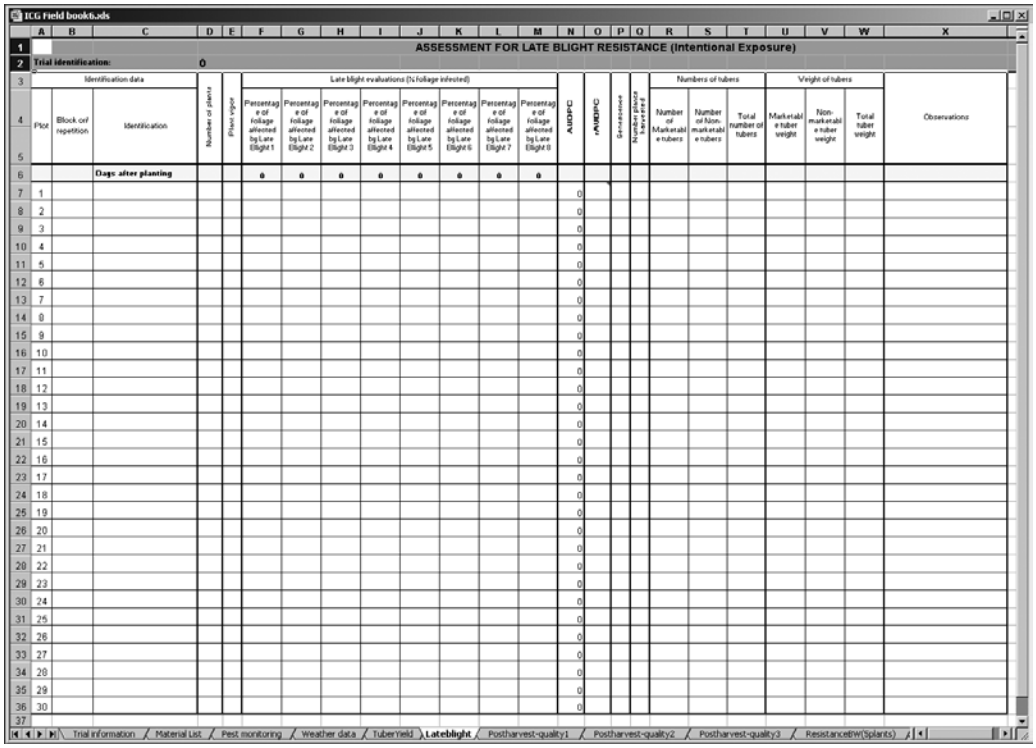
25																						
26																						
27																						
28																						
29																						
30																						

Sprouting degree: 1= 0.1cm (sprout length), 2= 0.5cm, 3=1.0cm, 4=1.5cm, 5=2cm

Trial information / Material List / Pest monitoring / Weather data / TuberYield / Lateblight / Postharvest-quality1 / Postharvest-quality2 / **Postharvest-quality3** / ResistanceBW(Splants)

I. Late blight worksheet: Assessment for late blight resistance (intentional exposure)



ICG Field book6.xls

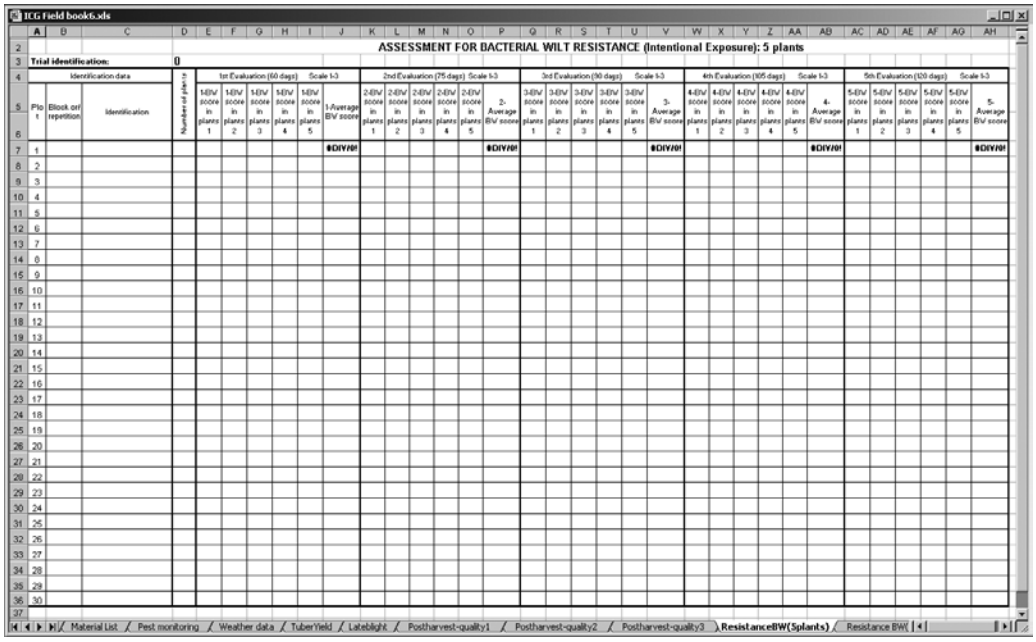
ASSESSMENT FOR LATE BLIGHT RESISTANCE (Intentional Exposure)

Trial identification: 0

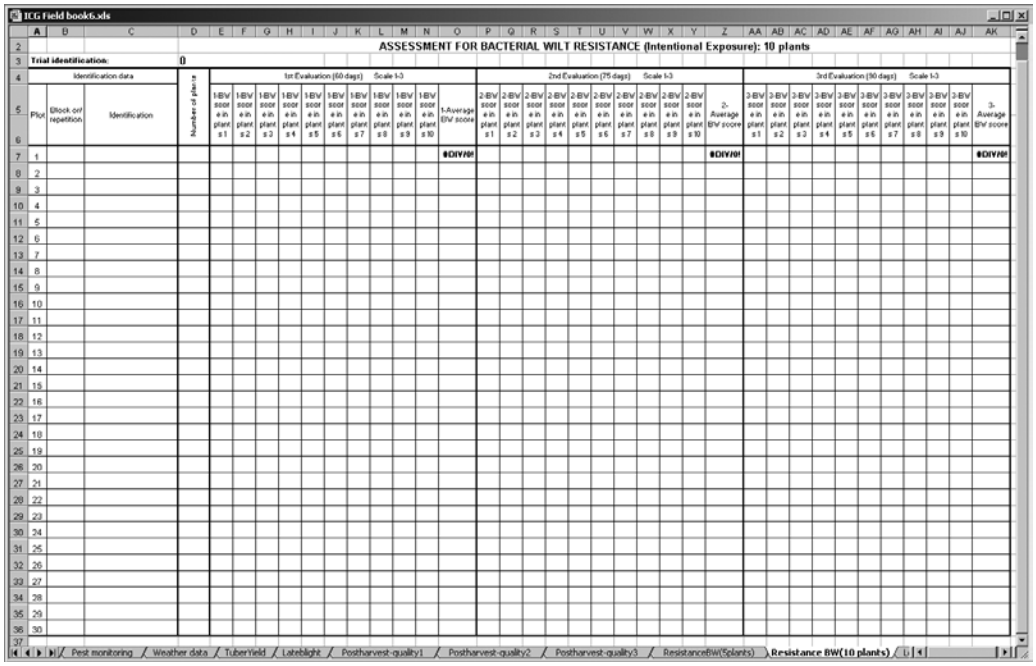
	Identification data					Late blight evaluations (% Foliage infested)												Numbers of tubers			Weight of tubers			
	Plot	Block or repetition	Identification	Number of plants	Plant vigor	Percentage of foliage affected by Late Blight 1	Percentage of foliage affected by Late Blight 2	Percentage of foliage affected by Late Blight 3	Percentage of foliage affected by Late Blight 4	Percentage of foliage affected by Late Blight 5	Percentage of foliage affected by Late Blight 6	Percentage of foliage affected by Late Blight 7	Percentage of foliage affected by Late Blight 8	AUDPC	rAUDPC	Senescence	Number plants harvested	Number of Marketable tubers	Number of Non-marketable tubers	Total number of tubers	Marketable tuber weight	Non-marketable tuber weight	Total tuber weight	Observations
			Days after planting			0	0	0	0	0	0	0	0											
7	1													0										
8	2													0										
9	3													0										
10	4													0										
11	5													0										
12	6													0										
13	7													0										
14	8													0										
15	9													0										
16	10													0										
17	11													0										
18	12													0										
19	13													0										
20	14													0										
21	15													0										
22	16													0										
23	17													0										
24	18													0										
25	19													0										
26	20													0										
27	21													0										
28	22													0										
29	23													0										
30	24													0										
31	25													0										
32	26													0										
33	27													0										
34	28													0										
35	29													0										
36	30													0										
37																								

Trial information / Material List / Pest monitoring / Weather data / TuberYield / Lateblight / Postharvest-quality1 / Postharvest-quality2 / Postharvest-quality3 / ResistanceBW(Splants)

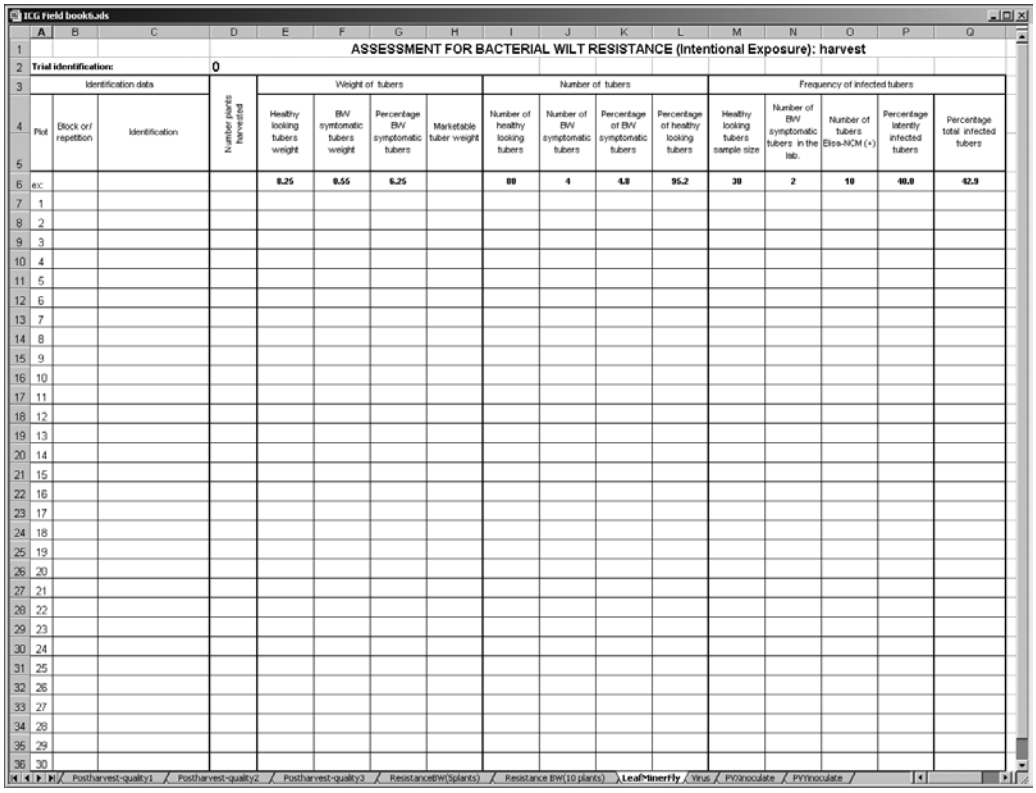
J. Resistance BW (5 plants) worksheet: Assessment for bacterial wilt (intentional exposure): 5 plants



K. Resistance BW (10 plants) worksheet: Assessment for bacterial wilt (intentional exposure): 10 plants



L. BW Harvest worksheet: Assessment for bacterial wilt (intentional exposure): harvest



ICG Field book6.xls

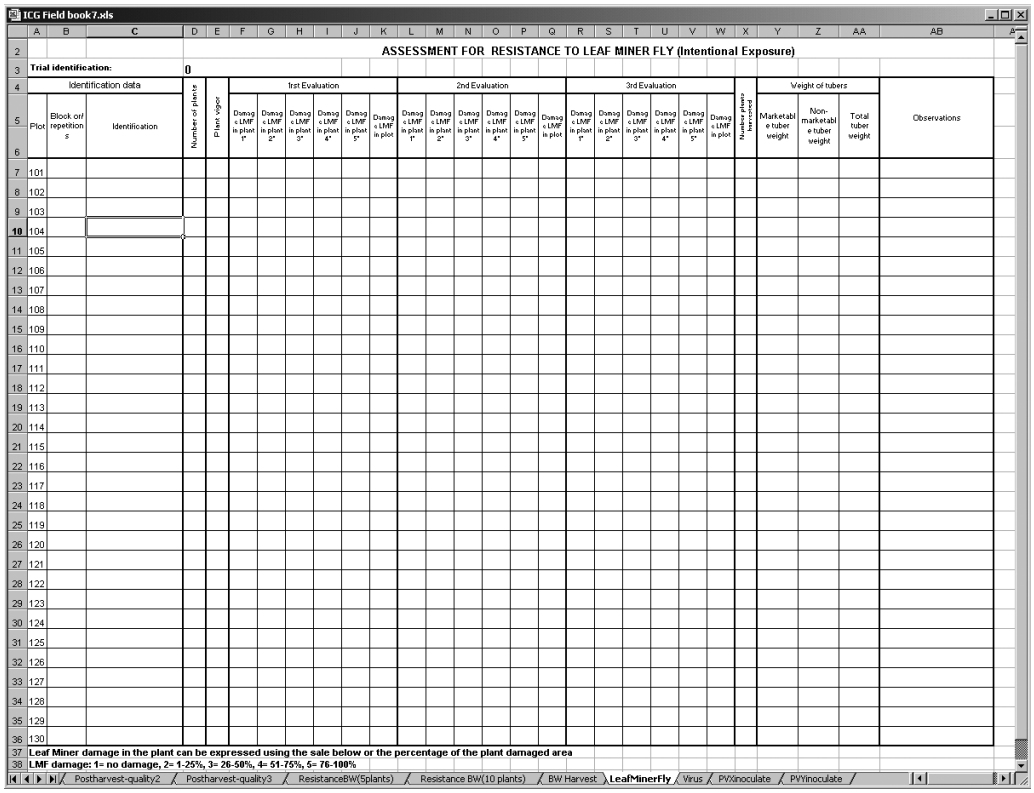
ASSESSMENT FOR BACTERIAL WILT RESISTANCE (Intentional Exposure): harvest

Trial identification: 0

	Identification data				Weight of tubers				Number of tubers				Frequency of infected tubers				
	Plot	Block or repetition	Identification	Number plants harvested	Healthy looking tubers weight	BW symtomatic tubers weight	Percentage BW symptomatic tubers	Marketable tuber weight	Number of healthy looking tubers	Number of BW symptomatic tubers	Percentage of BW symptomatic tubers	Percentage of healthy looking tubers	Healthy looking tubers sample size	Number of BW symptomatic tubers in the lab.	Number of tubers Elisa-NCM (+)	Percentage latently infected tubers	Percentage total infected tubers
6	exc				8.25	0.55	6.25		80	4	4.8	95.2	30	2	10	40.0	42.9
7	1																
8	2																
9	3																
10	4																
11	5																
12	6																
13	7																
14	8																
15	9																
16	10																
17	11																
18	12																
19	13																
20	14																
21	15																
22	16																
23	17																
24	18																
25	19																
26	20																
27	21																
28	22																
29	23																
30	24																
31	25																
32	26																
33	27																
34	28																
35	29																
36	30																

Postharvest-quality1 / Postharvest-quality2 / Postharvest-quality3 / ResistanceBW(5plants) / Resistance BW(10 plants) / LeafMinerFly / Virus / PVXinoculate / PYVinoculate

M. Leaf Miner Fly worksheet: Assessment for resistance to leaf miner fly (intentional exposure)



ICG Field book7.xls

ASSESSMENT FOR RESISTANCE TO LEAF MINER FLY (Intentional Exposure)

Trial identification: 0

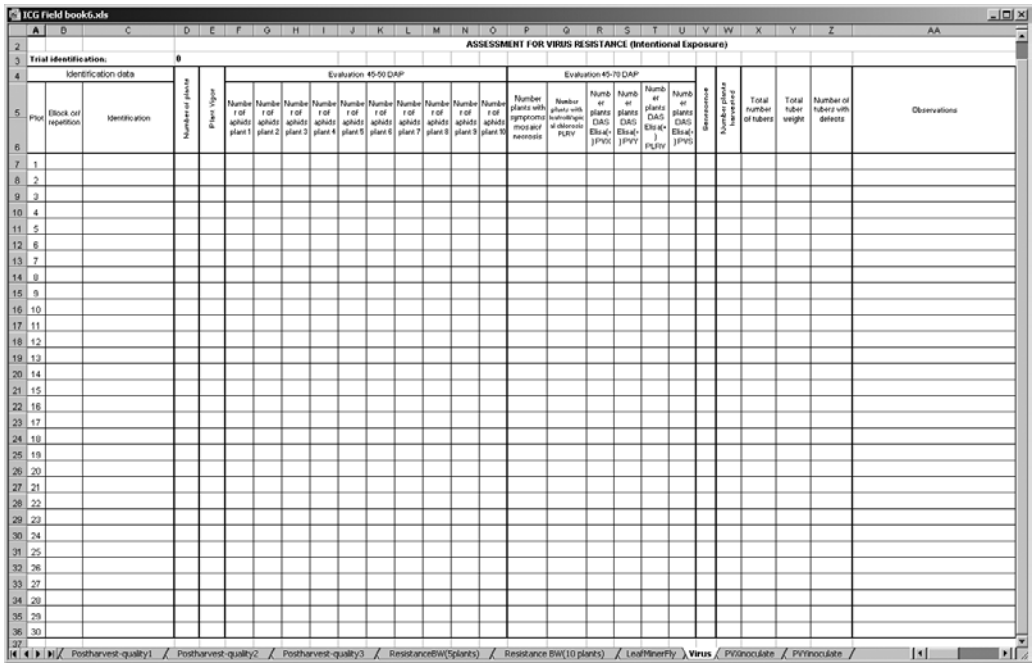
Identification data					1rst Evaluation						2nd Evaluation						3rd Evaluation							Weight of tubers			
Plot	Block or/ repetitions	Identification	Number of plants	Plant vigor	Damage LMF in plant 1*	Damage LMF in plant 2*	Damage LMF in plant 3*	Damage LMF in plant 4*	Damage LMF in plant 5*	Damage LMF in plot	Damage LMF in plant 1*	Damage LMF in plant 2*	Damage LMF in plant 3*	Damage LMF in plant 4*	Damage LMF in plant 5*	Damage LMF in plot	Damage LMF in plant 1*	Damage LMF in plant 2*	Damage LMF in plant 3*	Damage LMF in plant 4*	Damage LMF in plant 5*	Damage LMF in plot	Number plants harvested	Marketable tuber weight	Non-marketable tuber weight	Total tuber weight	Observations
101																											
102																											
103																											
104																											
105																											
106																											
107																											
108																											
109																											
110																											
111																											
112																											
113																											
114																											
115																											
116																											
117																											
118																											
119																											
120																											
121																											
122																											
123																											
124																											
125																											
126																											
127																											
128																											
129																											
130																											

Leaf Miner damage in the plant can be expressed using the sale below or the percentage of the plant damaged area

LMF damage: 1= no damage, 2= 1-25%, 3= 26-50%, 4= 51-75%, 5= 76-100%

Postharvest-quality2 / Postharvest-quality3 / ResistanceBW(5plants) / Resistance BW(10 plants) / BW Harvest / LeafMinerFly / Virus / PVXinoculate / PVYinoculate

N. Virus worksheet: Assessment for virus resistance (intentional exposure)


ICG Field book6.xls

ASSESSMENT FOR VIRUS RESISTANCE (Intentional Exposure)

Trial identification: 0

Identification data					Evaluation 45-50 DAP										Evaluation 45-70 DAP										
Plot	Block or repetition	Identification	Number of plants	Plant Vigor	Number of aphids plant 1	Number of aphids plant 2	Number of aphids plant 3	Number of aphids plant 4	Number of aphids plant 5	Number of aphids plant 6	Number of aphids plant 7	Number of aphids plant 8	Number of aphids plant 9	Number of aphids plant 10	Number plants with symptoms mosaic or necrosis	Number plants with leaf/stem or tuber necrosis PLRV	Number plants DAS Elisa(+) PVX	Number plants DAS Elisa(+) PVY	Number plants DAS Elisa(+) PLRV	Number plants DAS Elisa(+) PVS	Senescence	Number plants harvest	Total number of tubers	Total tuber weight	Number of tubers with defects	Observations
1																									
2																									
3																									
4																									
5																									
6																									
7																									
8																									
9																									
10																									
11																									
12																									
13																									
14																									
15																									
16																									
17																									
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30																									
31																									
32																									
33																									
34																									
35																									
36																									
37																									

Postharvest-quality1 / Postharvest-quality2 / Postharvest-quality3 / ResistanceBW(5plants) / Resistance BW(10 plants) / LeafMinerFly / Virus / PVXinoculate / PVYinoculate

O. PVX inoculate worksheet: Assessment for PVX virus resistance (greenhouse)

ICG Field book6.xls

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1				ASSESSMENT FOR PVX VIRUS RESISTANCE (Greenhouse)													
2	Trial identification:			0													
3				PVX extreme resistance test													
4	Identification data			Mechanical Inoculation (MI) date:						Graft Inoculation (GI) date:						Backtest (Positive / Negative)	Extreme Resistance Yes/No
5	Plot	Block or/ repetition	Identification	MI-Plant 1		MI-Plant 2		MI-Plant 3		GI-Plant 1		GI-Plant 2		GI-Plant 3			
6				Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test		
7	1																
8	2																
9	3																
10	4																
11	5																
12	6																
13	7																
14	8																
15	9																
16	10																
17	11																
18	12																
19	13																
20	14																
21	15																
22	16																
23	17																
24	18																
25	19																
26	20																
27	21																
28	22																
29	23																
30	24																
31	25																
32	26																
33	27																
34	28																
35	29																
36	30																

Postharvest-quality2 / Postharvest-quality3 / ResistanceBW(Splants) / Resistance BW(10 plants) / LeafMinerFly / Virus / PVXinoculate / PVYinocula

P. PVY inoculate worksheet: Assessment for PVY virus resistance (greenhouse)

ICG Field book6.xls

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1				ASSESSMENT FOR VIRUS RESISTANCE (Greenhouse)													
2	Trial identification:			0													
3				PVYextreme resistance test													
4	Identification data			Mechanical Inoculation (MI) date:						Graft Inoculation (GI) date:						Backtest (Positive / Negative)	Extreme Resistance Yes/No
5	Plot	Block or/ repetition	Identification	MI-Plant 1		MI-Plant 2		MI-Plant 3		GI-Plant 1		GI-Plant 2		GI-Plant 3			
6				Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test	Symptom	ELISA test		
7	1																
8	2																
9	3																
10	4																
11	5																
12	6																
13	7																
14	8																
15	9																
16	10																
17	11																
18	12																
19	13																
20	14																
21	15																
22	16																
23	17																
24	18																
25	19																
26	20																
27	21																
28	22																
29	23																
30	24																
31	25																
32	26																
33	27																
34	28																
35	29																
36	30																

Postharvest-quality3 / ResistanceBW(Splants) / Resistance BW(10 plants) / LeafMinerFly / Virus / PVXinoculate / **PVYinoculate**

Annex 3

French-frying score sheet

Cooking quality score sheet.xls

	A	C	D	E	F	G	H	I	J	K	L
1	FRENCH FRY SCORE SHEET										
2	Test Number:_____ Panelist _____ Date:_____										
3	For each sample, and for each quality factor, indicate your assessment by marking a cross (X) in the square opposite your assessment and vertically below the sample number. Assess external appearance for ALL samples at the beginning, before assessing other factors for each sample.										
4	Factor	Sample number									
5											
6	**External appearance (overall appearanace of the entire sample)**										
7	Excellent										
8	Very good										
9	Good										
10	Fair										
11	Poor										
12	**External color (overall appearanace of the entire sample)**										
13	Light, whitish										
14	Light golden										
15	Golden										
16	Slightly brown										
17	Dark										
18	**Internal color (break open the fries)**										
19	Bright, white, 'crystaline'										
20	Bright, white										
21	Off-white, 'opaque'										
22	Greyish										
23	Dark grey										
24	**Texture (mealiness) of outside strips**										
25	Crispy										
26	Moderately crispy										
27	Slightly crispy/soggy										
28	Moderately soggy										
29	Soggy										
30	**Texture (mealiness) of inside strips**										
31	Mealy										
32	Moderately mealy/soggy										
33	Sligthly mealy/soggy										
34	Soggy										
35	Very soggy										
36											
37	**Overall French fry quality**	0	0	0	0	0	0	0	0	0	0
38											

French fry / Cooking

Column B of the Excel document is hidden and contains a suggested weighing factor used to compute the overall French fry quality of each sample. Those numbers should not be seen by the taster in order to maintain the objectivity of the evaluation.

Annex 4

Cooking quality score sheet

Cooking quality score sheet.xls

	A	C	D	E	F	G	H	I	J	K	L
1	COOKING QUALITY SCORE SHEET										
2	Test Number:_____ Panelist _____ Date:_____										
3	For each sample, and for each quality factor, indicate your assessment by marking a cross (X) in the square opposite your assessment and vertically below the sample number. Assess appearance for ALL samples at the beginning. Then assess the other factors for each sample. Finally, when all assessments for all samples are completed, assess discoloration for ALL samples										
4	Factor	Sample number									
5											
6	**Appearance (on whole tuber)**										
7	Excellent										
8	Very good										
9	Good										
10	Fair										
11	Poor										
12	**Appearance (on mashed sample)**										
13	Excellent										
14	Very good										
15	Good										
16	Fair										
17	Poor										
18	**Texture**										
19	Extremely Mealy										
20	Very Mealy										
21	Moderately Mealy										
22	Slightly Mealy/Soggy										
23	Moderately Soggy										
24	Soggy										
25	Very Soggy										
26	**Off-flavor (on mashed sample)**										
27	None										
28	Slight										
29	Moderate										
30	Much										
31	Extreme										
32	**Sloughing (on mashed sample)**										
33	None										
34	Slight										
35	Moderate										
36	Much										
37	Extreme										
38	**Discoloration (on whole tuber)**										
39	None										
40	Slight										
41	Moderate										
42	Much										
43	Extreme										
44											
45	**Overall cooking quality**										

French fry | Cooking

Column B of the Excel document is hidden and contains a suggested weighing factor to compute the overall French fry quality of each sample. Those numbers should not be seen by the taster in order to maintain the objectivity of the evaluation.

Annex 5

Virus symptoms on potato

Virus symptoms	Abbr.	Viruses
Leafroll or rolling of basal leaves (rolled leaves are stiff and leathery)	LR	PLRV
Leafroll + chlorosis	LR + Ch	PLRV
Interveinal chlorosis (upper leaves) in S. tuberosum ssp andigena	Ch	PLRV
Mosaic (severe / mild)	M	PVY, PVX, PVS, PVA, APLV
Rugosity (always associated with mosaic)	R	PVY, PVA
Necrosis (leaf necrotic spots &/or stem streaks)	N	PVX, PVY
Stunting	St	PVY, PLRV
Mottle (mild to severe)	Mt	APMV, APLV
Aucuba or Calico (bright yellow markings: v-shapes, rings, blotches, fleckings)	Ca	PMTV, AMV, TRSV, PBRSV
Bright yellowing of veins (except primary veins)	YV	Potato Yellow Vein Disease