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Field assessment of resistance in potato to *Phytophthora infestans*

International Cooperators Guide

G.A. Forbes • W. Pérez • J. Andrade-Piedra

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Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the main biotic constraint to potato production worldwide. The present guide is designed to assist professionals and technicians in charge of evaluation trials designed to screen selected potato genotypes for resistance to this disease. The evaluation of breeding families, which is carried out under greenhouse or field conditions, can use the same methodology. The guide can help to organize trials, improve data collection and analysis and introduces new criteria for resistance measurement based on epidemiological principles. The International Potato Center (CIP) staff can share their late blight trial data through to the Global Trial Data Management System.

Phytophthora infestans, causal agent of late blight

Late blight of potato (and tomato) is caused by *Phytophthora infestans*. A recent classification scheme based on molecular analysis of the nuclear srRNA gene as well as ultra-structural data, places the genus *Phytophthora* in 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 by cellulose in cell walls. It is now clear that oomycetes are not related to the ascomycete and basidiomycete true fungi (10).

The asexual form of *P. infestans* is essentially an obligate parasite in nature. Mycelium may survive for short periods in crop debris, but in general the pathogen requires a living host (cultivated or wild) for long-term survival and cannot overwinter or overseason if no host is available. However, in some locations where sexual reproduction occurs, the resulting oospore can survive for months or years in the absence of living hosts (4,11). *P. infestans* can be cultivated on artificial media and can survive for indefinite periods in its vegetative state in the laboratory.

After a very long time during which *P. infestans* populations were considered to be only asexual, a 1984 report of A2 mating types in Western Europe was the first indication of new and dramatic developments in the pathogen populations (19,27). The analyses of a large number of dispersed local populations indicated, surprisingly, that the changes were not restricted to Western Europe, but were worldwide (20,22). These new migrations involved both mating types (A and A2) leading to the potential for sexual reproduction. 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 level 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 (5, 21). More information regarding the disease cycle, conducive weather, control, etc. is well documented in the literature (5,20,25,32,39).

Late blight symptoms on leaves

The disease first appears as water-soaked irregular pale green lesions mostly near the 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

Late blight also attacks the stem, where it can cause girdling and leaf wilting above the point of infection. Light to dark brown lesions on stems or petioles elongate and may completely encircle them (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 by secondary organisms and is not a direct consequence of late blight.

Resistance to late blight

There is general consensus that resistance to *P. infestans* could be classified into two phenotypes (two expressions in the field). The first is governed by single dominant genes with major effects and a clear, discontinuous segregation of progeny of a cross between resistant and susceptible potato genotypes. The second type of resistance is governed by several or many genes, called minor genes, with small cumulative effects and continuous distribution of resistant genotypes in progeny resulting from resistant by susceptible cross. Major gene resistance has also been described as vertical resistance, R-gene resistance, qualitative resistance, 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, race non-specific resistance, stable resistance, partial resistance, field resistance and rate-reducing resistance (7,12,37,38). Many varieties released as late blight resistant have rapidly become susceptible as the pathogen population evolves; examples are the cultivar Victoria in Uganda (known as Asante in Kenya) (12) and the cultivar Canchan in Peru (Table 1). The ephemeral nature of R-gene –mediated resistance and the difficulty of transferring quantitatively inherited resistance have rendered the identification and diffusion of durable resistance to late blight a difficult task (8,25).

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

Conducting late blight evaluation trials

The physical environment influences the development of an epidemic through effects on various phases of the pathogen's life cycle as the pathogen interacts with specific phases in the development of the host plant (6, 24, 33). Cool, wet weather with rainfall and ambient relative humidity (RH) above 90% and temperatures of 7 to 21 °C favor late blight development (1,5,34). Natural epidemics of *P. infestans* in the field can be used advantageously for screening large populations of potato genotypes for resistance to this disease (23).

Locations: screening sites

Knowledge about the pathogen population from the screening sites may be helpful for the interpretation of results. For example in Peru, two sites in the central highlands (Comas and Oxapampa) are used by CIP's late blight breeding program (31); the pathogen populations in those zones were formerly of the US-1 lineage (23,35,39), but they are both now dominated by the EC-1 lineage, which has been found to predominate in Ecuador (13), Colombia and Venezuela (14). Ultimately, the number of sites chosen, as well as the number of years of screening, also depends on logistics, human and financial resources.

One way to study phenotypic stability in crop performance trials is through the analysis of genotype by environment (G x E) interactions. G x E interaction can be studied temporally (two or more seasons at the same location) or spatially (several locations during the same season) or a combination of these (9) through the additive main effects and multiplicative interaction (AMMI) analysis (3).

Materials: Clones, controls and quality of planting material

- **Clones:** Breeding lines or potato varieties from local breeding programs or from CIP can be evaluated.
- **Controls:** A small number (1 to 5) of potato genotypes with known levels of resistance ranging from susceptible (scale value from 6 to 9) to highly resistant (scale value from 0 to 2) should be included in the evaluation trial, particularly if the evaluation is done across different locations or times. For the resistance scale described below, it is desirable to have one highly susceptible (scale value near 9) genotype among the controls. Preferably, this same genotype should be used in all evaluations.
- **Quality of planting material:** Uniform healthy tubers of the same origin for both advanced clones and controls should be used. It is advisable to plant tubers approximately equal in size for all genotypes evaluated in the trial.

- **Plot size:** Resistance to late blight can be evaluated in small plots (ca 5 plants). However, the larger plot (e. g., 4 X 4 m^2) the greater the resolution provided among materials being evaluated.
- **Agronomic management:** Fertilization, weed and pest control, as well as any other agricultural activities must be uniform for all plots of the trial and should conform to local agriculture practices.

Experimental design

The evaluation of clones should be done in a replicated trial using 3-4 repetitions. Late blight resistance can be evaluated in all classical experimental designs, such as completely randomized or a randomized block design.

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 sufficient. For inoculation to work, the inoculum can not dry out. Thus, inoculation must be done after dew formation or after a rain in the early evening. Alternatively, prior to inoculation, the field can be sprayed with overhead irrigation for a sufficiently long period to be sure all foliage is wet. It is best to inoculate plants at dusk so that the inoculum won't dry and to protect zoospores and sporangia from direct sunlight. The inoculum should be applied as evenly as possible in each plot with a hand-held, manually pumped sprayer (approximately 20 ml per plant) (12,17,18). Inoculation should be made on plants that have not yet reached flowering to allow time for disease development.

Another measure frequently used to improve the uniformity of disease across a field is to plant either one known susceptible genotype (scale value between 6 and 9) or both a susceptible and a moderately known resistant genotype (scale value between 4 and 5) of potato around the plots in order to produce continuous sources of inoculum. These additional genotypes are often referred to as "spreader rows" (Picture 5). Spreader rows may introduce other biases. For example, 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 sporangia released. To avoid this, spreaders can be planted between each row so that all rows receive an equal amount of inoculum, but this doubles the size of the experiment.

Picture 5. Spreader rows planted around the plot

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 detect. Under these conditions, it is advisable to protect plants with fungicides until they are of sufficient size for evaluation. Generally, a contact fungicide (propineb or chlorotalonil) is applied until the plants are considered large enough for evaluation, i.e. when they reach 30% of their full-grown leaf area. Fungicide applications must stop at least three weeks before any inoculation is made.

Evaluations of disease severity

Disease severity is evaluated as the percentage of foliage area that is infected. This variable is recorded throughout the season and the date of each reading is also logged. Data are collected on each experimental unit (each clone or variety within each replication). Data may be registered by hand or with electronic devices (e.g., tablets) in order to shorten the time and reduce the cost of collecting data and further analysis.

When the number of the plants per plot is low, some researchers take severity readings on each plant. However, there is little evidence for any advantage to this process and it requires significantly more time. For that reason, CIP recommends simply taking data at the plot level. The first reading should be taken before disease initiation. Researchers must therefore be aware of the normal time of appearance of late blight in their location, and/or survey nearby fields for any symptoms. Data recording should start as soon as weather conditions become conducive to blight development; if symptoms are seen, readings should begin immediately.

The percentages of leaf area are used to calculate the area under the disease progress curve (AUDPC), therefore, constant time intervals among readings are not really crucial. If the disease is advancing quickly in susceptible genotypes, readings should be done frequently (every 7 days in cold areas, or every 3 to 4 days in warm and humid areas). If the disease is advancing slowly, the interval between readings can be longer (every 10 to 14 days). The objective is to have readings at low, medium and high levels of disease in all genotypes, including susceptible ones.

When evaluating late blight, most researchers visually estimate the percentage of total leaf area that is affected by the disease. This is done by simply comparing the green and non-green portions (assuming late blight is the only or dominant foliage disease). Thus, one mentally estimates the percent of infected foliage in the plot.

This is a standard procedure and generally works well, especially when readings are integrated into a measure like the AUDPC. Nonetheless, the estimation of percent infection is subject to several sources of error.

The readings are then integrated into a measure like the area under the development progress curve (AUDPC) (16) (see below).

Source of errors when estimating the percentage infection

Estimation of percentage infection is, nonetheless, subject to several sources of error (15).

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 routine 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 (29). The use of logarithmically-based scales does not necessarily correct this bias and thus simple percentage infected area is advised (16).

3) In general, it is better if all readings for a trial are taken by an evaluator with expertise in disease assessment in order to maintain the same degree of accuracy and precision in the disease assessments (6). 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 digital recorder which can hide the previous reading. If paper is used to take disease readings, the color should be chosen to avoid reflection of sunlight. The best time of the day to evaluate disease severity is in the morning or in cloudy conditions.

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 AUDPC to summarize the disease readings into one synoptic measure (18). The AUDPC is calculated from the estimated percentages of leaf area affected recorded at different times during the epidemic.

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

The AUDPC is frequently calculated using the midpoint formula (6):

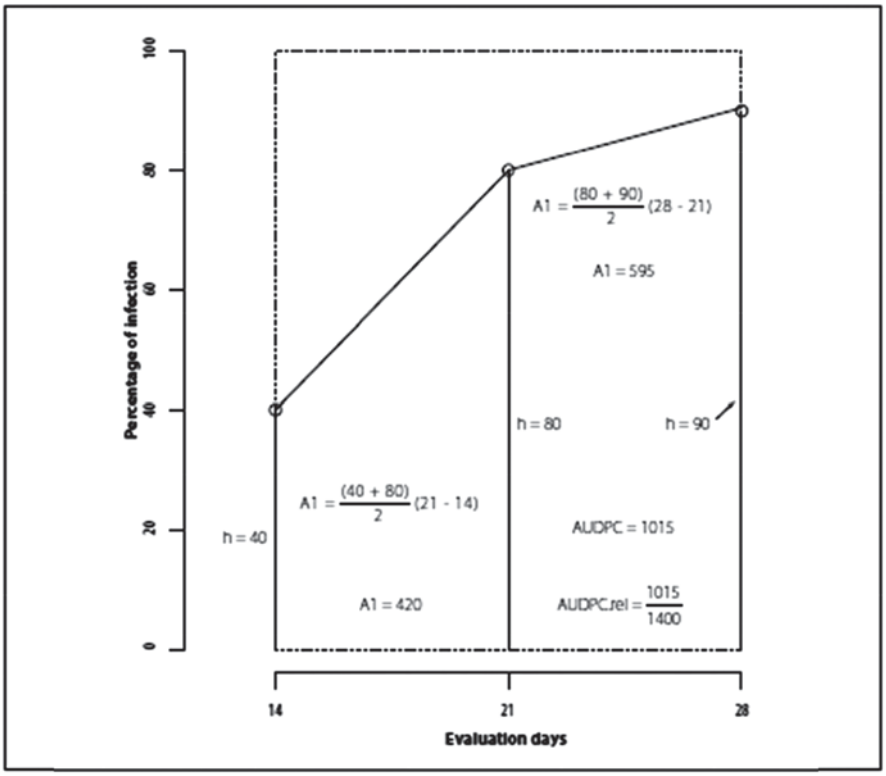
$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where "t" is the time of each reading, "y" is the percentage 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. DataCollector (https://research.cip.cgiar.org/confluence/display/GDET4RT/Downloads) also calculates the AUDPC.

Figure 1. Graphic representation of the AUDPC



Data analysis

The AUDPC values, as well as percentage infection values, can be analyzed using analysis of variance (ANOVA) after exploration of the data through simple statistics such as means, standard errors, frequency distributions and box plots. Multiple means comparisons tests (e.g., Dunnett) can also be applied (36). The analysis of residuals is recommended to test the validity of the model and assess homogeneity of the variances (36).

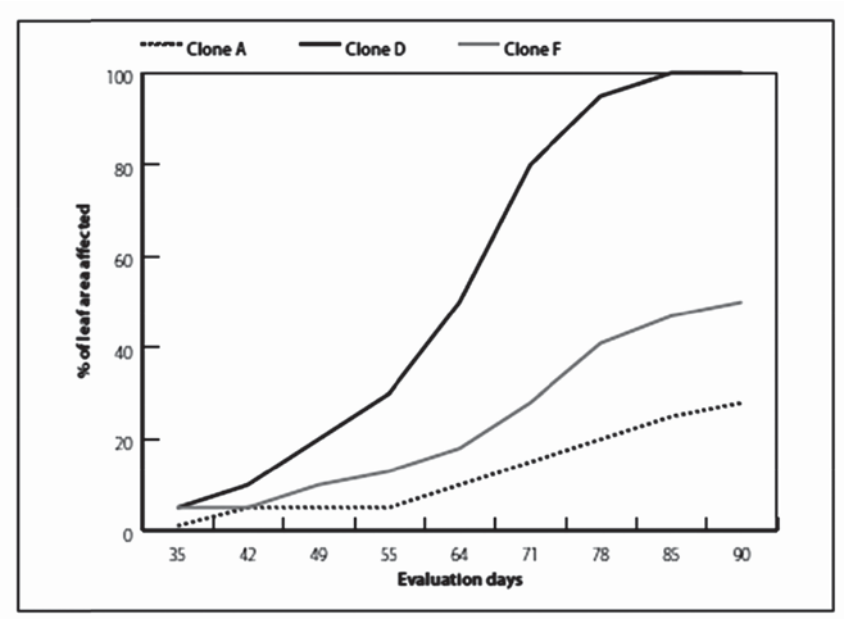
The AUDPC and percentage infection are considered pseudo-quantitative variables with hierarchy and can be analyzed without transformation (30) .

If yield has been evaluated in addition to the AUDPC, the correlation between yield and the AUDPC can be calculated with the Spearman method (28). The Spearman method works by ranking each observation within each replication and the Spearman rank correlation is calculated on the ranked values (28). A coefficient close to 1.0 indicates a good correlation between yield and the AUDPC (i.e., overall disease severity).

Data interpretation

The AUDPC is a variable which estimates the amount of disease across the season. The AUDPC is expressed in % days (that is, the accumulation of daily percentage infection values) and is interpreted directly without transformation. The higher the AUDPC, the more disease in the genotype. It is often helpful to plot the percentage leaf area infected versus the evaluation date ("disease progress curve") to get a better idea of how genotypes perform in the experiment (Figure 2).

Figure 2. Disease severity curves of three potato clones



Getting around common problems associated with the AUDPC

Timing the readings

Problem: The selection of resistant potato genotypes is frequently based on the AUDPC (26). However, the same AUDPC value can result from an early-starting, slow-progressing infection or from a late-starting, fast-progressing infection; therefore AUDPC values do not provide information on the type of resistance present in the genotypes, nor on their potential durability. For this reason it is also advisable to examine disease progress curves (2).

Solution: It is important to start the readings when disease begins, ideally even before the first symptoms appear. Generally, it is not relevant to continue the readings after the susceptible materials reach 100% disease severity. While an AUDPC value can be calculated from two data points, greater accuracy is achieved with each additional observation, although under some conditions very accurate AUDPC values can be derived from two data points (30).

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. If disease is typically non-uniform in a location, inoculation should help.

Dealing with incomparability across experiments

Problem: The AUDPC per se should not be used for comparing potato genotypes across experiments. Furthermore, the AUDPC units as indicators of resistance or susceptibility are not easily interpretable. For example, an AUDPC value of 2043 may arise from a moderately resistant genotype grown under conditions conducive to severe infection, or it could also arise from a highly susceptible genotype grown under conditions not conducive to severe infection.

Solution: In an effort to standardize the AUDPC, researchers often use the relative AUDPC (rAUDPC) (18). The rAUDPC is calculated by dividing the AUDPC by the "maximum potential AUDPC."

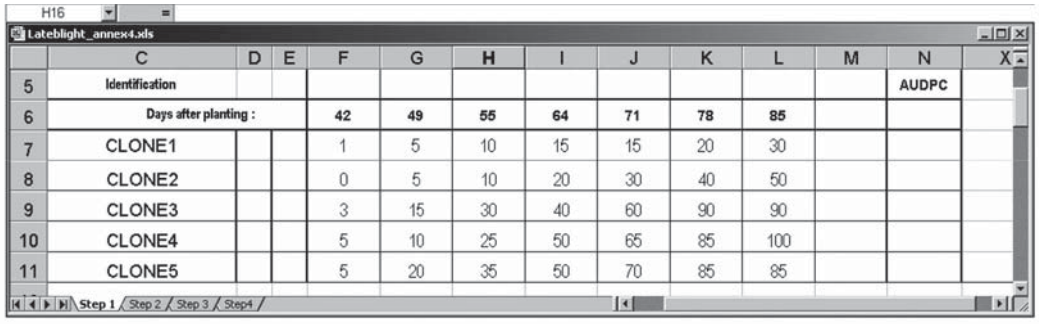
The maximum potential AUDPC is simply the AUDPC a genotype would have if it had 100% infection at all readings. The maximum potential AUDPC is represented by the dotted line in Figure 1 and is calculated by multiplying the total number of days between the first and last readings by 100.

The rAUDPC is also not the best measure for comparing results across different experiments for the same reasons explained above for AUDPC. For this task, CIP recommends the resistance scale described below.

Calculating the 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 =

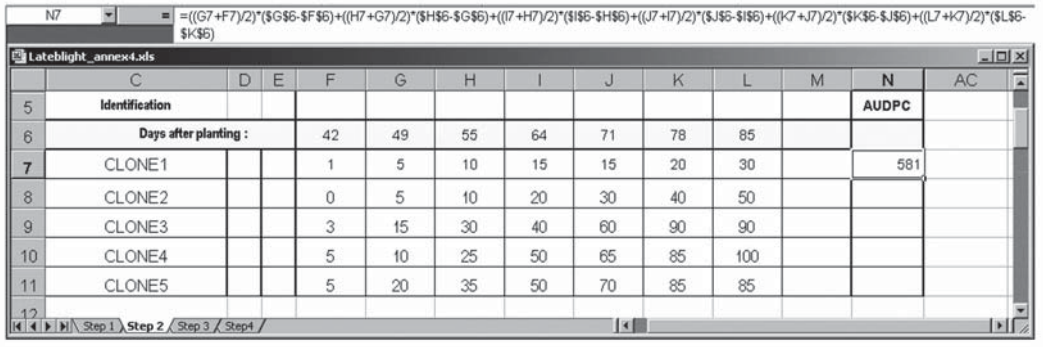
Lateblight_annex4.xls

	C	D	E	F	G	H	I	J	K	L	M	N	X
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)



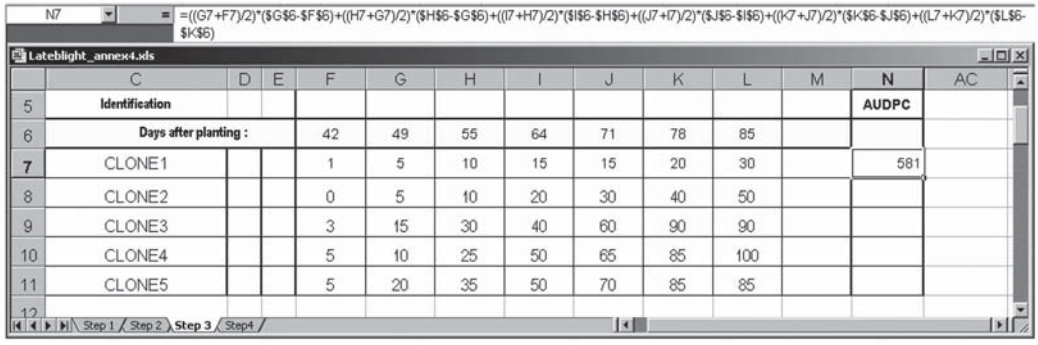
N7 =((G7+F7)/2)*(G6-F6)+((H7+G7)/2)*(H6-G6)+((I7+H7)/2)*(I6-H6)+((J7+I7)/2)*(J6-I6)+((K7+J7)/2)*(K6-J6)+((L7+K7)/2)*(L6-K6)

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 cell N7.



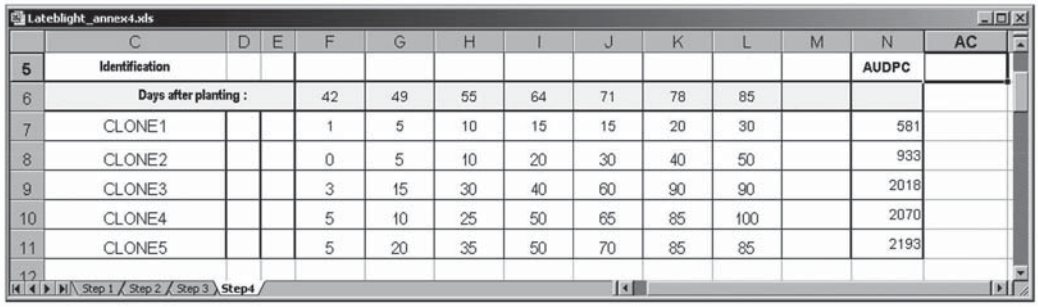
N7 =((G7+F7)/2)*(G6-F6)+((H7+G7)/2)*(H6-G6)+((I7+H7)/2)*(I6-H6)+((J7+I7)/2)*(J6-I6)+((K7+J7)/2)*(K6-J6)+((L7+K7)/2)*(L6-K6)

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

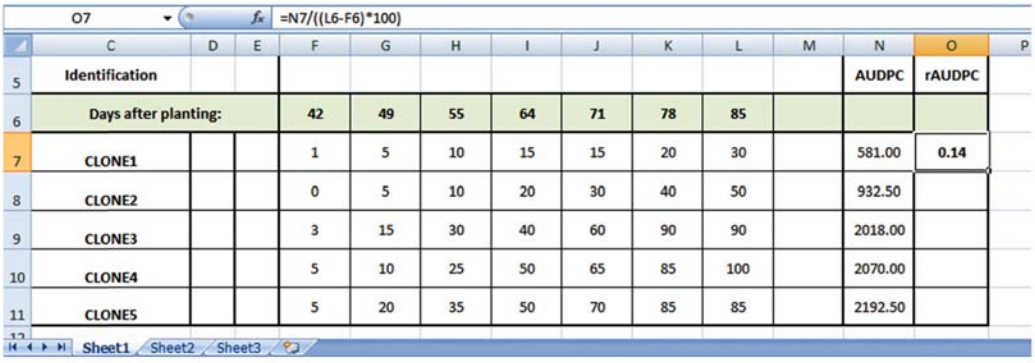
Calculating the rAUDPC with Microsoft Excel

The rAUDPC can also be calculated using statistical analysis or spreadsheet programs. The same spreadsheet prepared to find the AUDPC is used.

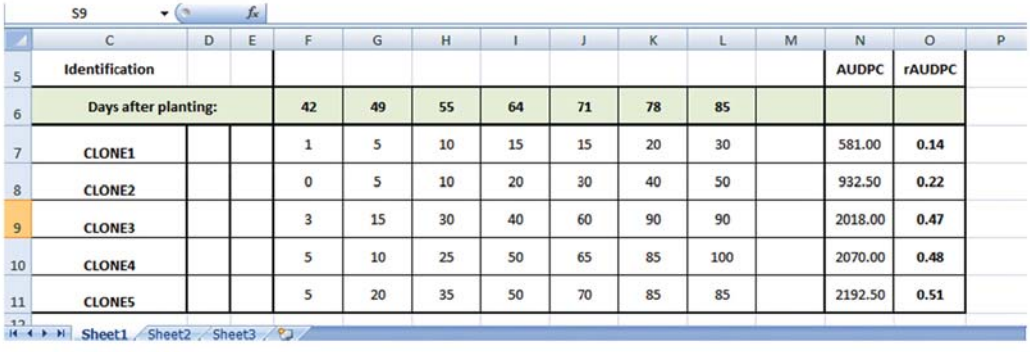
Step 1: Locate the cursor in cell O7, which corresponds to the rAUDPC of clone 1, and enter the following formula:

rAUDPC =N7/((L6-F6)*100)

now press Enter, and the rAUDPC 0.14 will appear in cell O7.



Step 2: Copy the formula of the O7 cell to other cells from O8 to O11, but be careful to maintain the values of the last (cell L6) and first (cell F6) readings in every formula copied.



An alternative for calculating the rAUDPC is to place the data from the last and first readings in the formula. In the example, the last reading is 85 days (cell L6) and the first reading is 42 days (cell F6).

rAUDPC =N7/((85-42)*100)

O7 =N7/((85-42)*100)

	C	D	E	F	G	H	I	J	K	L	M	N	O	P
5	Identification											AUDPC	rAUDPC	
6	Days after planting:			42	49	55	64	71	78	85				
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14	
8	CLONE2			0	5	10	20	30	40	50		932.50		
9	CLONE3			3	15	30	40	60	90	90		2018.00		
10	CLONE4			5	10	25	50	65	85	100		2070.00		
11	CLONE5			5	20	35	50	70	85	85		2192.50		

Sheet1 Sheet2 Sheet3

Calculating resistance (susceptibility) scale values to *Phytophthora infestans* for potato genotypes

In most parts of the world there is no standard system for measuring the degree of resistance to *Phytophthora infestans* in potato genotypes. One generally finds that genotypes are classified as resistant, moderately resistant or susceptible. This classification can be helpful but is very limited in comparing genotypes across environments and is too crude to provide useful information for fungicide management. This situation is particularly problematic in developing countries, because until recently no scale was available for short-day conditions, as is the case of highland tropics (39). To address this problem, Yuen and Forbes (39) proposed a simple scale (0 to 9) that can be calculated from AUDPC or rAUDPC values; however to use this scale it is neccesary to use a susceptible cultivar as a common reference genotype in all experiments that are to be compared.

The resistance scale values are found using the following equation:

$$Sx = Sy \frac{Dx}{Dy}$$

where Sy and Dy represent, respectively, the assigned susceptibility scale value and observed disease measure (AUDPC or rAUDPC) for the standard genotype, and Sx and Dx represent, respectively, the calculated susceptibility scale value and observed disease measurement for the genotype in question. In essence, one divides the assigned susceptibility value of the control by the resistance measure of the control (e.g. AUDPC or rAUDPC) to get a constant. This can then be multiplied by the resistance measure of each target cultivar to get the susceptibility value of that genotype.

Calculating the scale value with Microsoft Excel

Step 1: The same spreadsheet that was used to calculate the AUDPC and rAUDPC is used here. Select a susceptible cultivar used in all experiments. Locate the cursor in cell P12 and assign the appropriate value of the scale of susceptibility (generally 8 or 9) to this susceptible cultivar (example: Desiree, Bintje, Tomasa Condemayta, Diacol Capiro , etc.).

In the example, Desiree is the cultivar selected as susceptible (cell C12) and value 9 was assigned (cell P12).

P12 | 9

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
5	Identification											AUDPC	rAUDPC	Highest scale value	
6	Days after planting:			42	49	55	64	71	78	85					
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14		
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22		
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47		
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48		
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51		
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	
13															

Step 2: The assigned value is divided by the rAUDPC calculated for same cultivar and the value obtained will be used as a constant for following procedures. In the examples, the value assigned for Desiree is 9 (cell P12) and the rAUDPC calculated for this variety in this experiment is 0.65 (cell O12).

In the spreadsheet, locate the cursor in cell Q12 and enter the following formula:

C=P12/O12

Press Enter and the constant 13.91 appears in cell Q12.

Q12 | =P12/O12

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
5	Identification											AUDPC	rAUDPC	Highest scale value	Constant
6	Days after planting:			42	49	55	64	71	78	85					
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14		
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22		
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47		
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48		
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51		
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	13.91
13															

Step 3: The constant obtained must be multiplied for each of the rAUDPC values of the other genotypes to obtain the respective scale values.

In the spreadsheet, locate the cursor in cell R7 and enter the following formula:

Susceptible scale value for clone 1 = O7*Q12

Press Enter and the scale value 1.88 appears in cell R7.

R7 =O7*Q12

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
5	Identification											AUDPC	rAUDPC	Highest scale value	Constant	Susceptibility scale values
6	Days after planting:			42	49	55	64	71	78	85						
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14			1.88
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22			
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47			
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48			
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51			
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	13.91	
13																

Step 4: Copy the formula of the R7 cell to other cells from R8 to R12, but be careful to maintain the value of the constant (cell Q12) in every formula copied.

R12 =O12*Q12

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
5	Identification											AUDPC	rAUDPC	Highest scale value	Constant	Susceptibility scale values
6	Days after planting:			42	49	55	64	71	78	85						
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14			1.88
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22			3.02
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47			6.53
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48			6.70
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51			7.09
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	13.91	9.00
13																

An alternative for calculating the scale value for each cultivar is to place the value of the constant in the formula:

In the spreadsheet, locate the cursor in cell R7 and enter the following formula:

Susceptible scale value for clone 1 = O7*13.91

Press Enter and the scale value 1.88 appears in cell R7.

R7 =O7*13.91

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
5	Identification											AUDPC	rAUDPC	Highest scale value	Constant	Susceptibility scale values
6	Days after planting:			42	49	55	64	71	78	85						
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14			1.88
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22			
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47			
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48			
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51			
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	13.91	
13																

Copy the formula of the R7 cell to other cells from R8 to R12. Notice that the scale value assigned to Desiree is 9 after this calculation, which is similar to the same as the value previously assigned.

R12 =O12*13.91

	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
5	Identification											AUDPC	rAUDPC	Highest scale value	Constant	Susceptibility scale values
6	Days after planting:			42	49	55	64	71	78	85						
7	CLONE1			1	5	10	15	15	20	30		581.00	0.14			1.88
8	CLONE2			0	5	10	20	30	40	50		932.50	0.22			3.02
9	CLONE3			3	15	30	40	60	90	90		2018.00	0.47			6.53
10	CLONE4			5	10	25	50	65	85	100		2070.00	0.48			6.70
11	CLONE5			5	20	35	50	70	85	85		2192.50	0.51			7.09
12	Desiree			15	35	45	65	85	100	100		2782.50	0.65	9	13.91	9.00
13																

Examples of use of susceptibility scale values

Potato cultivar Canchan-INIA developed at CIP in the late 1970s and released in Peru in 1990 as resistant to late blight, but pathogenic strains of *P. infestans* were rapidly selected as Canchan became more popular and it is now considered very susceptible throughout Peru (Table 1). Despite its susceptibility to late blight, Canchan has maintained its popularity in Peru as an important cash crop because of its stable yield performance, earliness, and market demand. The resistance scale is useful to visualize the increase in susceptibility of Canchan caused by changes in the pathogen population.

Table 1.- Historical behavior of cultivar Canchan (CIP 380389.1) in the Peruvian highlands.

Agroecological zone (Altitude range m.a.s.l.) [a]			
Huánuco [b,c,d] (2500 – 2700)	**Cajamarca [a,b,c] (2500 – 2800)**	**Junín [a,b,c,e] (2600 – 2800)**	**Pasco [a,b,c,e] (1700 – 1900)**
1983 – 1984 (1)	1990 – 1991 (1)	1998 – 1999 (9)	1998 – 1999 (9)
1984 – 1985 (1)	1991 – 1992 (0)	1999 – 2000 (9)	
1985 – 1986 (1)	1991 – 1992 (0)	1999 – 2000 (9)	
1986 – 1987 (1)	1992 – 1993 (1)		
1987 – 1988 (2)	1993 – 1994 (2)		
1988 – 1989 (1)	1994 – 1995 (2)		
	1996 – 1997 (1)		
	1990 – 1991 (1)		

[a] Zones used by CIP as screening sites due to their conducive conditions for late blight development.

[b] The value between parentheses indicates scale value of cultivar Canchan in an experiment carried out in the agroecological zone during the respective growing season.

[c] Cultivar Tomasa Condemayta was used as susceptible and it was assigned the highest scale value = 9.

[d] Scale value analyzed from data published by Egusquiza,R.(ed.) 1984 -1990. Sistema Nacional de Evaluación de Recursos Genéticos 1984 -1990. Instituto Nacional de Investigación y Promoción Agropecuaria (INIPA) – Universidad Nacional Agraria (UNA). Lima, Peru.

[e] Scale value analyzed from data collected by CIP Late Blight Breeding Program.

Cultivar Yungay was selected as susceptible control for CIP's breeding program in Peru. Other varieties, such as Tomasa Condemayta and Chata Blanca, have higher scale values (Table 2) but these are so susceptible that they often reach high levels of disease too early in the growing season to accurately estimate the rAUDPC and AUDPC values for the experimental genotypes.

Table 2. Determination of scale value of potato cultivar Yungay used in CIP's Late Blight Breeding Program as susceptible variety.

Agroecological zone	Growing season	Susceptible variety used as control[a]	Scale value calculated for cultivar Yungay[b]
	1998 – 1999	Canchan (9)	8
	1999 – 2000	Canchan (9)	7
	2000 – 2001	Canchan (9)	6
Comas, Junin	2004 – 2005	Chata Blanca (9)	6
	2005 – 2006	Chata Blanca (9)	7
	2006 – 2007	Tomasa Condemayta (9)	8
	2007 – 2008	Chata Blanca (9)	8
	1998 – 1999	Canchan (9)	7
Oxapampa, Pasco	2005 – 2006	Chata Blanca (9)	5
	2008 – 2009	Chata Blanca (9)	8
	2009 – 2010	Desiree (9)	7

[a] The highest scale value assigned to the susceptible variety used in the experiment.

[b] Scale value analyzed from data collected by CIP Late Blight Breeding Program.

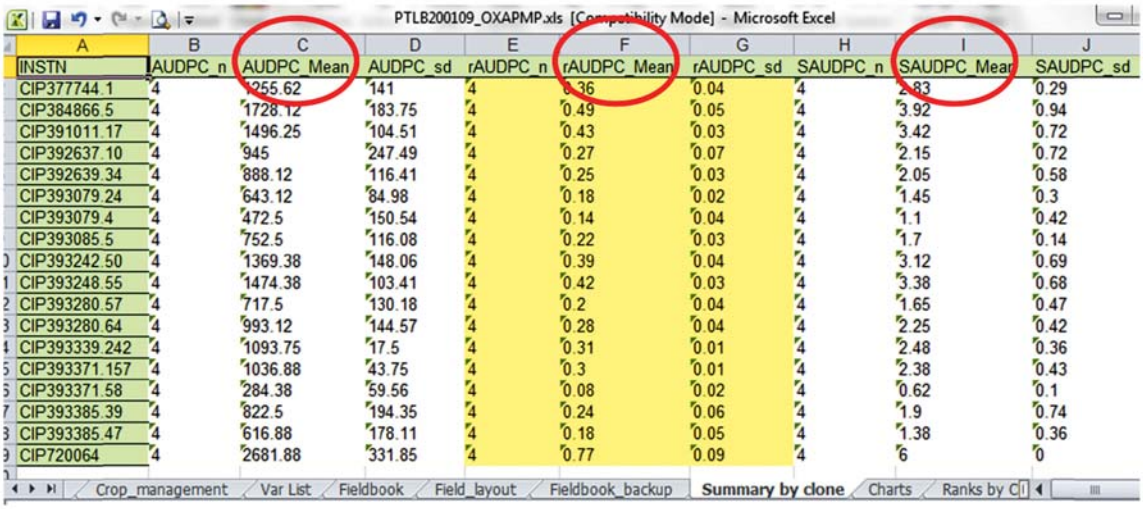
The same varieties tested in different locations may obtain different scale values for many reasons (Table 3). For example, small differences of one or even two scale values may result from environmental effects or even experimental artifacts. Small or large differences may also result from potato genotypes containing effective R genes for which pathogenic strains appear at different times in the season in different locations.

Table 3. Scale values obtained for selected Peruvian potato varieties in two screening sites during the same growing season (2010 – 2011); the Tomasa Condemayta variety was used as control and was assigned a value of 9.

	Scale values	
Varieties	**Oxapampa (1813 m.a.s.l.)**	**Paucartambo (2480 m.a.s.l.)**
Chucmarina	0	0
Venturana	0	0
Serranita	1	0
UNICA	2	5
Amarilis	3	6
Capiro	4	7
Liberteña	4	7
Perricholi	5	6
Yungay	5	7
Chaska	7	8
Tomasa	9	9
Canchan	9	8

CIPs Global Database of field trials for potato and sweetpotato

CIP, together with partners, is promoting the use of DataCollector, a software that helps to standardize data and ensure data quality (12). It is part of the International Potato Center's Global Data Management System (34) and assists researchers in data analysis by automatically calculating the AUDPC, rAUDPC and susceptibility scale values (Figure 3).

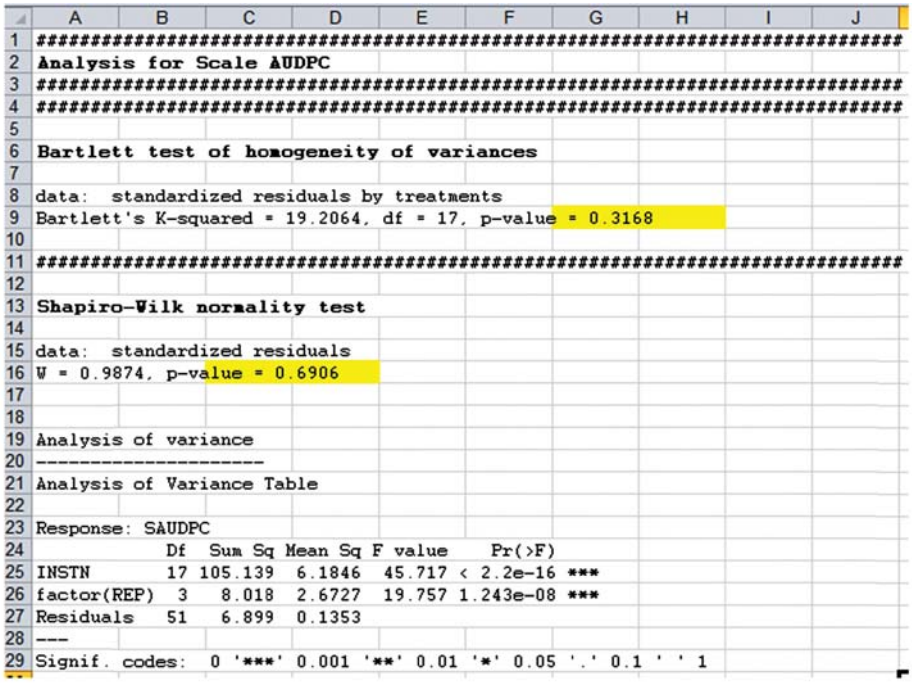


	A	B	C	D	E	F	G	H	I	J
	INSTN	AUDPC_n	AUDPC_Mean	AUDPC_sd	rAUDPC_n	rAUDPC_Mean	rAUDPC_sd	SAUDPC_n	SAUDPC_Mean	SAUDPC_sd
	CIP377744.1	4	1255.62	141	4	0.36	0.04	4	2.83	0.29
	CIP384866.5	4	1728.12	183.75	4	0.49	0.05	4	3.92	0.94
	CIP391011.17	4	1496.25	104.51	4	0.43	0.03	4	3.42	0.72
	CIP392637.10	4	945	247.49	4	0.27	0.07	4	2.15	0.72
	CIP392639.34	4	888.12	116.41	4	0.25	0.03	4	2.05	0.58
	CIP393079.24	4	643.12	84.98	4	0.18	0.02	4	1.45	0.3
	CIP393079.4	4	472.5	150.54	4	0.14	0.04	4	1.1	0.42
	CIP393085.5	4	752.5	116.08	4	0.22	0.03	4	1.7	0.14
0	CIP393242.50	4	1369.38	148.06	4	0.39	0.04	4	3.12	0.69
1	CIP393248.55	4	1474.38	103.41	4	0.42	0.03	4	3.38	0.68
2	CIP393280.57	4	717.5	130.18	4	0.2	0.04	4	1.65	0.47
3	CIP393280.64	4	993.12	144.57	4	0.28	0.04	4	2.25	0.42
4	CIP393339.242	4	1093.75	17.5	4	0.31	0.01	4	2.48	0.36
5	CIP393371.157	4	1036.88	43.75	4	0.3	0.01	4	2.38	0.43
6	CIP393371.58	4	284.38	59.56	4	0.08	0.02	4	0.62	0.1
7	CIP393385.39	4	822.5	194.35	4	0.24	0.06	4	1.9	0.74
8	CIP393385.47	4	616.88	178.11	4	0.18	0.05	4	1.38	0.36
9	CIP720064	4	2681.88	331.85	4	0.77	0.09	4	6	0
0										

Figure 3. EXCEL sheet produced by Data Collector showing AUDPC, rAUDPC and susceptibility scale values.

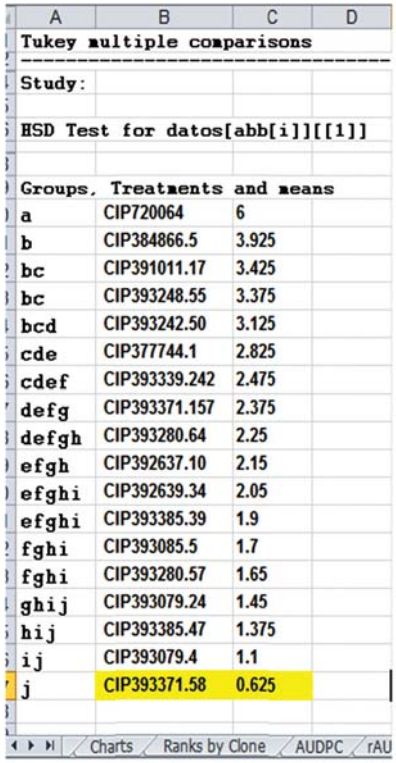
DataCollector also performs statistical analysis based on late blight resistance scale values or a number of other parameters (Figures 4 and 5). The software is still under development, but ultimately it will give access to genotype pedigrees, trial metadata, and experimental data from potato and sweetpotato trials. A User's Manual can be downloaded free of charge from: https://research.cip.cgiar.org/confluence/display/GDET4RT/Home

Figure 4. Statistics analysis performed with Data collector using scale values.



```
##############################################################################
Analysis for Scale AUDPC
##############################################################################
##############################################################################

Bartlett test of homogeneity of variances

data:  standardized residuals by treatments
Bartlett's K-squared = 19.2064, df = 17, p-value = 0.3168

##############################################################################

Shapiro-Wilk normality test

data:  standardized residuals
W = 0.9874, p-value = 0.6906


Analysis of variance
--------------------
Analysis of Variance Table

Response: SAUDPC
             Df  Sum Sq Mean Sq F value    Pr(>F)
INSTN        17 105.139  6.1846  45.717 < 2.2e-16 ***
factor(REP)   3   8.018  2.6727  19.757 1.243e-08 ***
Residuals    51   6.899  0.1353
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 5. Means comparison performed with Data Collector using scale values.



```
Tukey multiple comparisons
--------------------------------

Study:

HSD Test for datos[abb[i]][[1]]

Groups, Treatments and means
a    CIP720064      6
b    CIP384866.5    3.925
bc   CIP391011.17   3.425
bc   CIP393248.55   3.375
bcd  CIP393242.50   3.125
cde  CIP377744.1    2.825
cdef CIP393339.242  2.475
defg CIP393371.157  2.375
defgh CIP393280.64  2.25
efgh CIP392637.10   2.15
efghi CIP392639.34  2.05
efghi CIP393385.39  1.9
fghi CIP393085.5    1.7
fghi CIP393280.57   1.65
ghij CIP393079.24   1.45
hij  CIP393385.47   1.375
ij   CIP393079.4    1.1
j    CIP393371.58   0.625
```

Charts | Ranks by Clone | AUDPC | rAU

Literature cited

1. **Agrios, G. N.** 1989. Fitopatología. México: Editorial Limusa.

2. **Andrivon, D., Pelle, R., and Ellisseche, D.** 2006. Assessing resistance types and levels to epidemic diseases from the analysis of disease progress curves : Principles and application to potato late blight. American Journal of Potato Research 83:455–461.

3. **Annicchiarico, P.** 1997. Additive main effects and multiplicative interaction (AMMI) analysis of genotype-location interaction in variety trials repeated over years. Theoretical and Applied Genetics 94:1072–1077.

4. **Bashi, E., Ben-Joseph, Y., and Rotem, J.** 1982. Inoculum potential of *Phytophthora infestans* and the development of potato late blight epidemics. Phytopathol. 72:1043–1047.

5. **Beaumont, A.** 1947. The dependence on the weather of the dates of outbreak of potato blight epidemics. Trans. Br. Mycol. Soc. 31:45–53.

6. **Campbell, C. L., and Madden, L. V.** 1990. Introduction to Plant Disease Epidemiology. New York: John Wiley & Sons.

7. **Collinge, D. B., and Slusarenko, A. J.** 1987. Plant gene expresion in response to pathogens. Plant Molecular Biology 9:389 – 410.

8. **Cooke, L., Schepers, H., Hermansen, A., Bain, R., Bradshaw, N., Ritchie, F., Shaw, D., Evenhuis, A., Kessel, G., Wander, J., Andersson, B., Hansen, J., Hannukkala, A., Nærstad, R., and Nielsen, B.** 2011. Epidemiology and integrated control of potato late blight in Europe. Potato Research 54:183–222.

9. **Cotes, J. M., Nuñez, C. E., Martinez, R., and Estrada, N.** 2002. Analyzing genotype by environment interaction in potato using yield-stability index. American Journal of Potato Research 79:211–218.

10. **Dick, M. W.** 1995. Sexual reproduction in the Peronosporomycetes (chromistan fungi). Canadian Journal of Botany 73(Suppl. 1):5712–5724.

11. **Drenth, A., Janssen, E. M., and Govers, F.** 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. Plant Pathology (Oxford) 44:86–94.

12. **Forbes, G. A.** 2012. Using Host Resistance to Manage Potato Late Blight with Particular Reference to Developing Countries. Potato Research 55:205–216.

13. **Forbes, G. A., Escobar, X. C., Ayala, C. C., Revelo, J., Ordoñez, M. E., Fry, B. A., Doucett, K., and Fry, W. E.** 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. Phytopathology 87:375–380.

14. **Forbes, G. A., Goodwin, S. B., Drenth, A., Oyarzún, P., Ordoñez, M. E., and Fry, W. E.** 1998. A global marker database for *Phytophthora infestans*. Plant Disease 82:811–818.

15. **Forbes, G. A., and Jeger, M. J.** 1987. Factors affecting the estimation of disease intensity in simulated plant structures. Journal of Plant Diseases and Protection 94:113–12O.

16. **Forbes, G. A., and Korva, J. T.** 1994. The effect of using a Horsfall-Barratt scale on precision and accuracy of visual estimation of potato late blight severity in the field. Plant Pathology 43:675–682.

17. **Forbes, G. A., Trillos, O., Turkensteen, L., and Hidalgo, O.** 1993. Field inoculation of potatoes with *Phytophthora infestans* and its effect on the efficiency of selection for quantitative resistance in the plants. Fitopatología 28:117–120.

18. **Fry, W. E.** 1978. Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68:1650–1655.

19. **Fry, W. E., Grünwald, N. J., Cooke, D. E. L., McLeod, A., Forbes, G. A., and Cao, K.** 2009. Population genetics and population diversity of *Phytophthora infestans*. In Oomycete Genetics and Genomics: Biology, Interactions and Reserch Tools, New York: Wiley-Blackwell, p. 139–162.

20. **Garrett, K. A., and Mundt, C. C.** 2000. Host diversity can reduce potato late blight severity for focal and general patterns of primary inoculum. Phytopathology 90:1307–1312.

21. **Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E.** 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in Northern North America. Phytopathology 85:473–479.

22. **Goodwin, S. B., Sujkowski, L. S., and Fry, W. E.** 1995. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85:669–676.

23. **Gopal, J., and Singh, B.** 2003. Screening potato for resistance to late blight (*Phytophthora infestans*) under field conditions. Potato Research 46:47–56.

24. **Harrison, J. G.** 1992. Effects of the aerial environment on late blight of potato foliage - a review. Plant Pathology 41:384–416.

25. **Haverkort, A. J., Struik, P. C., Visser, R. G. F., and Jacobsen, E.** 2009. Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora Infestans*. Potato Res. 52:249–264.

26. **Haynes, K. G., and Weingartner, D. P.** 2004. The use of area under the disease progress curve to assess resistance to late blight in potato germplasm. American Journal of Potato Research 81:137–141.

27. **Hohl, H. R., and Iselin, K.** 1984. Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. Transactions of the British Mycological Society 83:529–530.

28. **Hollander, M., and Wolfe, D. A.** Nonparametric Statistical Methods. 2nd ed.

29. **Horsfall, J. G., and Barratt, R. W.** 1945. An improved grading stystem for measuring plant disease. Phytopathology 35:655.

30. **Jeger, M. J., and Viljanen-Rollinson, S. L. H.** 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics 102:32–36.

31. **Perez, W., Gamboa, S., Coca, M., Raymundo, R., Hijmans, R. J., and Nelson, R.** 1999. Characterization of *Phytophthora infestans* populations in Peru. Lima, Peru: International Potato Center (CIP).

32. **Rotem, J., Cohen, Y., and Putler, J.** 1971. Relativity of limiting and optimum inoculum loads, wetting durations, and temperatures for infection by *Phytophthora infestans*. Phytopathology 61:275–278.

33. **Rotem, J., Palty, J., and Lomas, J.** 1970. Effects of sprinkler irrigation at various times of the day on development of potato late blight. Phytopathology 839–843.

34. **Simon, R., Salas, E., Eyzaguirre, R., Hualla, V., de Haan, S., and Bonierbale, M.** 2012. Desarrollo de un "software estadistico" para estandarización y chequeo de calidad de datos del campo en raíces y tubérculos (GDET4RT) para el mejoramiento de cultivos. 12th International Conference, ALAP, Uberlandia Brazil. Available at: http://www.papaslatinas.org/.

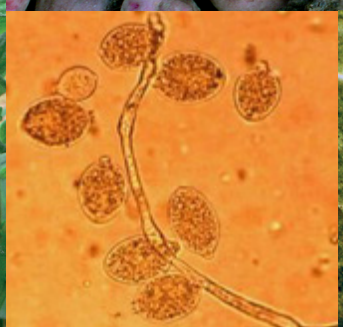
35. **Tooley, P. W., Therrien, C. D., and Ritch, D. L.** 1989. Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. Phytopathology 79:478–481.

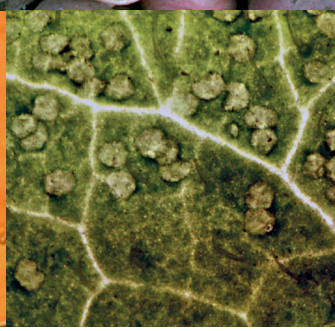
36. **Upton, G., and Cook, I.** 2008. A Dictionary of Statistics. 2nd ed. United Kingdom: Oxford University Press.

37. **Vanderplank, J. E.** 1956. Horizontial (polygenic) and Vertical (oligogenic) resistance against blight. American Potato Journal 42:306.

38. **Wale, S., Plat, H. W., and Cattlin, N.** 2008. Diseases, Pests and Disorders of Potatoes: A Colour Handbook. Manson Publishing Ltd.

39. **Yuen, J. E., and Forbes, G. A.** 2009. Estimating the level of susceptibility to *Phytophthora infestans* in potato genotypes. Phytopathology 99:783–786.





The International Potato Center (known by its Spanish acronym CIP) is a research-for-development organization with a focus on potato, sweetpotato, and Andean roots and tubers. CIP is dedicated to delivering sustainable science-based solutions to the pressing world issues of hunger, poverty, gender equity, climate change and the preservation of our Earth's fragile biodiversity and natural resources.

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