Potato Res. 19 (1976): 91-107

A laboratory method for measuring the degree of attack by *Phytophthora infestans**

V. UMAERUS[1] and D. LIHNELL[2]

[1] The Swedish Seed Association, S-268 00 Svalov, Sweden
[2]Formerly at The National Swedish Plant Protection Institute, S-171 07 Solna 7, Sweden

Accepted for publication: 28 July 1975

Zusammenfassung, Résumé p. 106

Summary

A method is described based on quantitative measurements of infection efficiency (IE), lesion growth (LES) and conidia production (CON). The three parameters have been used to calculate the leaf area destroyed by blight in order to illustrate varietal differences in disease progress and to correlate the findings with field assessments. The method has also been used as a replacement or supplement of field readings in experiments designed for studies of fungicidal treatments.

Introduction

Evaluation of the degree of attack by late blight (*Phytophthora infestans* (Mont.) de Bary) on the potato has been based almost exclusively on field observation, whether the objective has been to study varietal differences in resistance or the effect of fungicidal treatments or other factors influencing the disease.

Such field experiments depend on the natural spread of the disease, which irrespective of the spontaneous or artificial start of the epidemic, early or late, is to a large extent influenced by the weather conditions. In some areas blight epidemics are unpredictable and costly experiments may be carried out without results.

When testing for resistance, a difficulty arises from the presence of *R*- genes in an increasing number of cultivars. They mask the effect of non race-specific resistance, the evaluation of which is usually of greater importance to the breeder. Artificial introduction of races able to attack the *R*- genotypes is sometimes less successful because of the dominance in quality or pathogenicity of a more simple field race. In some areas the use of complex races may not be allowed by the authorities unless they have been isolated within that area. The interpretation of field data is also difficult because of interplot interference (James, 1973).

This paper describes a laboratory test, developed with the intention of replacing or supplementing field readings. The test is based on quantitative measurements of

* Communication from the Swedish Seed Association, No 422.

infection frequency, lesion growth or size and conidia production. Leaf samples for evaluating the method have been collected from a number of field experiments designed for studies of fungicidal treatments (methods of application, dosage response, test of chemicals) and resistance of different cultivars to blight.

Material and methods

Infection frequency

A quantitative inoculator (Fig. 1) was built as originally designed by Schein (1964). For several reasons (see Umaérus, 1969a) zoospores have been used as infection units instead of sporangia. Zoospores are, however, delicate in structure, sensitive to metal ions (Björling & Sellgren, 1955), and apparently to physical stress, resulting, for example, from high pressure or high velocity through small apertures.

Initial trials with a de Vilbiss atomizer model 15 often gave a high mortality of the zoospores at a pressure of 3 kg/cm^2, which was necessary to give a good spray. This atomizer, which has a metal spray nozzle, was replaced with a whole glass atomizer as illustrated in Fig. 2. The aperture of the outer tube has an inner

Fig. 1. The quantitative inoculator with timer (left), atomizer on a magnetic stirrer, and stand with replaceable target frames with holes of different shapes and sizes.

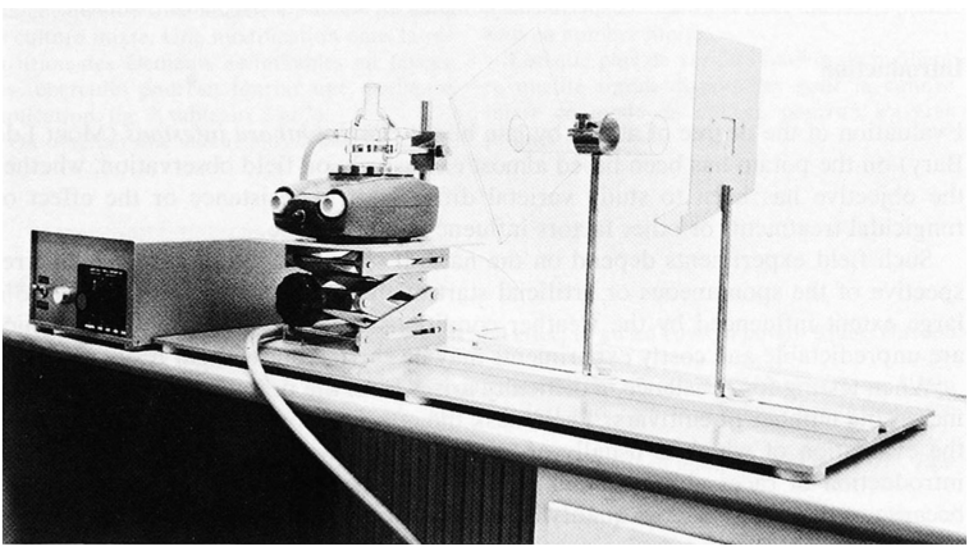


Abb. 1. Quantitativinokulator mit Zeitnehmer (links), Zerstäuber auf einem magnetischen Rührapparat und Stand mit auswechselbaren Scheibenrahmen mit Löchern von verschiedenen Formen und Grössen.

Fig. 1. Inoculateur quantitatif avec horloge (à gauche), atomiseur sur agitateur magnétique et monté avec un système de cibles amovibles percées de trous de différentes formes et dimensions.

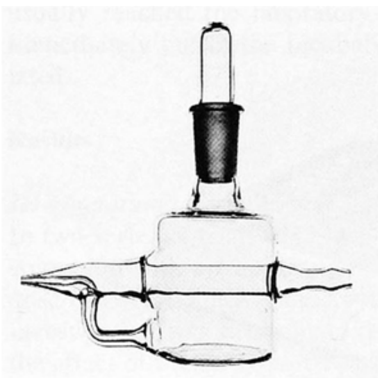


Fig. 2. The atomizer.

Abb. 2. Der Zerstäuber.
Fig. 2. L'atomiseur.

diameter of 1.8 mm. The inner tube has an outer diameter of 1.3 mm and inner diameter of 0.8 mm and is carefully centred in the aperture of the outer tube. To facilitate alignment the atomizer can be moved in all directions. An air pressure of 0.5-1.0 kg/cm^2 was optimal for a good spray.

Deposition of spores was monitored by agar-coated Petri dishes with a millimeter lattice of negative transparant film covering an area of 1 cm^2 moulded into the agar. After 15-20 h of incubation the spores were killed with alcoholic vapour as a fixative.

Inoculation of leaflets were made on a discrete target area of 1 cm^2.

Inoculated leaflets were incubated in plastic boxes lined with plastic foam and a sheet of tissue paper, which was soaked wet, and on the top of that a plastic net to support the leaves (Fig. 3). The box was covered with a plate of Vipolon plastic and incubated in a constant temperature room at + 15 °C.

Primary infections were counted 3 days after inoculation under a microscope with a magnification $\times 20$.

Data were transformed according to a formula of Kleczkowski (1949) before statistical analysis. The formula was programmed into a computer, which after analysis of variance and calculating means, detransformed those data.

As suggested by Schein (1964) the data are expressed in terms of infection efficiency (IE) with the following definition:

$$IE = \frac{\text{number of lesions/cm}^2}{\text{number of spores/cm}^2}$$

For easy comparison of different series of experiments IE has been calculated per 1000 deposited zoospores expressed as IE^{1000}. Each experiment is also reported in transformed data of number of infections based on the current number of zoospores deposited.

Fig. 3. Box for incubation of detached leaflets.

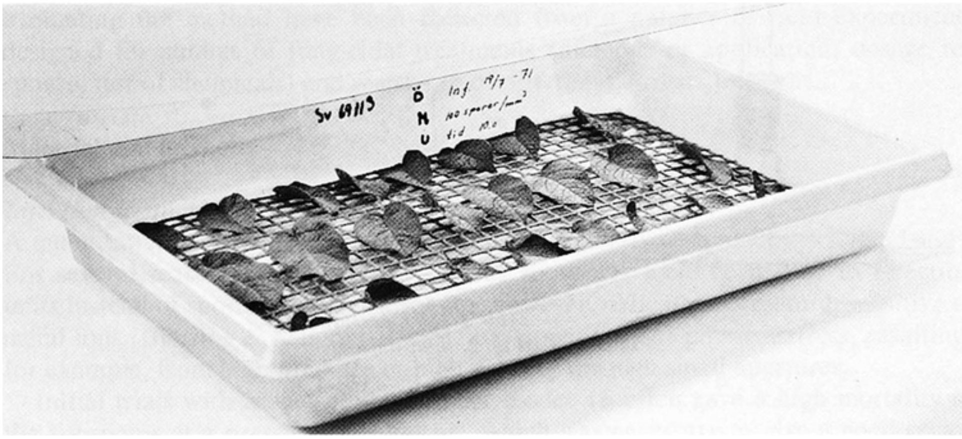


Abb. 3. Schale für die Inkubation der gepflückten Blättchen.
Fig. 3. Incubateur de folioles détachés.

Lesion size

The diameter of each lesion has been measured in mm along the axis parallel to the main vein of the leaflet on the 4th day after inoculation. Originally the 1-cm^2 target of the spray of the inoculator was used as initial of each lesion. This was abandoned because of difficulties with bacterial infections and measurements of lesion size were made on leaves inoculated by a small drop of spore suspension.

Conidia production

Conidia were collected on the 5th day after inoculation by shaking 10 leaflets in 20 ml of a 10% alcohol solution. The samples were stored in a refrigerator at +6 °C in closed vials until they were counted.

The conidia were counted in an electronic particle counter of the type Celloscope 302 as described for use in nematode work (Carlsson, 1969). The orifice tube used had an aperture with a diameter of 190 µm. The sample was suspended in 0.9% sodium chloride at a concentration of 100-2000 conidia/ml, which is the range most suitable for counting. Each sample was divided in two aliquots, which were run separately through the counter.

Sampling

For each sample twenty leaflets were picked at random from the centre of each plot in the field trials. The leaflets should be fully developed and have good turgidity. Terminal leaflets were avoided. Three such samples were taken representing three leaf positions: top, intermediate and bottom, and marked accordingly. Samples were taken in the morning and stored in a cool room until transport, usually by express mail. Each sample was packed in a small plastic bag closed with a stapler. Samples usually reached the laboratory at Svalöv the following morning, when they were immediately put in the incubating boxes, sprinkled with distilled water and inoculated.

Results

IE in relation to application and dosage of fungicides

In two series of trials the effect of fungicidal application was studied, ground spray compared with air application, and normal dose in comparison with a 50 % lower dose of two commonly used fungicides when ground sprayed. The objective of this investigation was to evaluate the efficiency of the quantitative inoculator and thus the effect of the different treatments will only be discussed in relation to this aspect.

Results in Table 1 and 2 refer to the IE when the lower leaf surface was inoculated. In both series of experiments inoculation of the upper leaf surfaces gave no infections in leaves from treated plots and in leaves from check plots about 10 times less infections than when infected on the lower leaf surface. The experiments were convincing that the fungicidal treatments, irrespective of way of application or dosage, were effective on the upper leaf surface.

Table 1. IE^{1000} of lower leaf surface and manganese deposit (mg $\times 10^{-2}$/100 cm^2 leaf area) resulting from ground spraying and aerial application.
Cultivar Dianella; fungicide Mancozeb; 1971.

Treatment[1]	13 July		3 August		10 August	18 August	23 August
	IE^{1000}	Mn	IE^{1000}	Mn	IE^{1000}	IE^{1000}	Mn
Control, unsprayed[2]	14.8	3.06	14.9	2.65	14.7	27.9	3.49
Ground spray[3]	12.3	16.12	0.0	17.95	0.0	4.9	23.04
Aircraft application, low volume[4], 40 l/ha	7.5	24.15	0.0	25.57	0.0	1.2	36.10
Aircraft application, high volume[5], 80 l/ha	7.1	14.55	0.0	22.06	0.0	3.5	33.17
Time of spraying[6]	8 July	19 July	29 July		9 August	19 August	

[1] *Verfahren - Traitement* [2] *Kontrolle, nicht gespritzt - Témoin, non traité;* [3] *Bodenspritzung - Pulvérisation terrestre;* [4] *Besprühung durch Flugzeug, kleine Menge - Application aérienne bas volume;* [5] *Besprühung durch Flugzeug grosse Menge - Application aérienne volume élevé;* [6] *Zeitpunkt der Spritzung - Epoque de traitement*

Tabelle 1. IE^{1000} der unteren Blattoberfläche und Mangan-Belag (mg $\times 10^{-2}$/100 cm^2 Blattfläche), die sich aus der Bodenspritzung und der Behandlung aus der Luft ergeben. Sorte Dianella; Fungizid Mancozeb; 1971.

Tableau 1. IE^{1000} de la surface de feuille la plus basse et dépôt de manganèse (mg $\times 10^{-2}$/100 cm^2 de surface de feuille) résultant de pulvérisations terrestre et aérienne. Variété Dianella; fongicide Mancozèbe; 1971.

Table 2. IE^{1000} of lower leaf surface at two levels of fungicidal treatment, 1.0-2.0 and 2.0-4.0 kg/ha, applied in successively increased dosages in 4-5 ground sprays. Cultivar Bintje.

Treatment	1971		1972									
			laboratory assessment[2]								field assessment[3]	
	17 Aug.		1 Aug.		2 Aug.		14 Aug.		22 Aug.		% defoliation[4]	% infected tubers[5]
	A*	B*	A	B	A	B	A	B	A	B		
Control, unsprayed[6]	56.5	1.44	38.4	1.37	23.4	1.37	13.6	1.35	10.8	1.31	< 8.0	17.4
Fungiman, 1.0-2.0 kg	23.5	1.25	23.2	1.31	17.4	1.30	9.8	1.30	5.2	1.19	< 0.2	3.5
Fungiman, 2.0-4.0 kg	19.5	1.21	16.0	1.23	9.9	1.22	8.8	1.27	4.2	1.16	< 0.1	2.5
De Zäta M-45, 1.0-2.0 kg	21.5	1.23	23.6	1.30	13.5	1.26	9.6	1.29	7.2	1.23	< 0.1	3.0
De Zäta M-45, 2.0-4.0 kg	7.5	1.09	12.0	1.19	9.3	1.21	7.8	1.26	3.2	1.11	< 0.1	1.7
LSD ($P < 0.05$)		0.14		0.04		0.05		0.03		0.05		
Percentage error[7]		11.4		7.9		8.2		5.3		8.7		
F value, treatment[8]		12.1***		60.1***		15.3***		20.8***		41.8***		
F value, leaf position[9]		—		28.9***		—		6.0*		46.7***		
Dosage[10], zoospores/cm²		200		250		325		535		500		

*A = number of infections/cm² per 1000 spores (IE^{1000}) - *Anzahl Infektionen/cm² pro 1000 Sporen (IE^{1000}) - Nombre d'infections/cm² par 1000 spores (IE^{1000}).*
B = transformed data (according to Kleczkowski, 1949), based on original counts - *Transformierte Werte (nach Kleczkowski, 1949), basierend auf Originalzählungen - Données transformées (selon Kleczkowski, 1949) basées sur les comptages originaux.*

[1] *Verfahren - Traitement;* [2] *Beurteilung im Labor - Evaluation au laboratoire;* [3] *Feldbeurteilung - Evaluation au champ;* [4] *Blattfall - Destruction de feuilles;* [5] *Befallene Knollen - Tubercules malades;* [6] *Kontrolle, nicht gespritzt - Témoin, non traité;* [7] *Fehlerprozent - Coefficient de variation;* [8] *F-Wert, Verfahren - Valeur de F, traitement;* [9] *Plazierung der Blätter - Position des feuilles;* [10] *Dosierung - Dosage*

Tabelle 2. IE^{1000} der unteren Blattoberfläche bei zwei Stufen der Fungizid-Anwendung 1.0-2.0 und 2.0-4.0 kg/ha. Dosierung in 4-5 Bodenspritzungen successive erhöht. Sorte Bintje.
Tableau 2. IE^{1000} de la surface de feuille la plus basse à deux niveaux de traitement fongicide, 1.0-2.0 et 2.0-4.0 kg/ha, appliqués en doses successivement croissantes en 4-5 pulvérisations terrestres. Variété: Bintje.

The IE when inoculating the lower leaf surface revealed, however, significant differences between treatments. Aircraft application seemed more efficient than ground spray, also manifested by the amount of manganese deposited (Table 1). On 10 August, the day after the 4th spraying, the protection was complete but slightly deteriorated by the end of that interval. The normal dosage of fungicide was significantly more effective than the lower dosage but there was no evidence of any difference between the two chemicals (Table 2). Field assessment could only recognize differences between the unsprayed control and the treated plots.

IE in relation to potato cultivar (IE_v)
There were significant differences in IE between different cultivars (Tables 3 and 4). One selection, SvUL 72156, has an outstandingly low IE, followed by three other selections. All have *S. demissum* in their pedigree and were selected at the seedling stage as resisters requiring a long minimum inoculation access period (Umaerus, 1969b).

IE_v and level of fertilizer application
Table 5 illustrates the interaction between cultivar and level of fertilizer application with regard to IE. No significant varietal difference was found at 400 kg per ha of NPK, while cv. Bintje had a significantly higher IE than cv. Grata at 1200 kg. The two cultivars seemed to react differently to nutrition: Bintje had more infections at 1200 kg than at 400 kg, while the reverse was true in the case of Grata. When comparison is made with field assessments it should be kept in mind that the laboratory assessment in this case is restricted to IE, other factors related to field resistance not being measured.

IE in relation to leaf position
As expected, in the case of fungicidal treatments, the lower leaves of the plants had more infections than leaves at the top. The analysis of variance in Table 2 indicates that significant differences are found with leaf positions. In all cases the bottom leaves had a higher IE. This might not be due only to a less effective application of the fungicide at the bottom of the plant canopy, as results from the unsprayed controls and from the cultivar tests indicate that the lower leaves in certain cultivars have more infections than the upper leaves.

From a total of 121 comparisons of leaf positions representing 11 cultivars, 3 leaf positions (top, intermediate and low) and weekly samplings over a period of 6 weeks (4 cultivars observed for 2 years) only 27 (= 22 %) comparisons gave significant differences in IE. In the majority of cases (23 comparisons) a lower leaf had a higher IE than leaves at a higher leaf position.

IE in relation to growth stage
A general trend in most cultivars both in 1971 and 1972 was a decrease in IE during the beginning of August from a higher level at the start of sampling in July

Table 3. IE^{1000} of five cultivars inoculated on the lower leaf surface, 1971.

Cultivar[1]	15 July		22 July		29 July		9 August		12 August		Mean[2]
	A*	B*	A	B	A	B	A	B	A	B	A
Sv 69113	41.5	1.35	20.5	1.18	43.9	1.46	33.5	1.31	28.3	1.27	33.5
Prominent	53.0	1.40	16.0	1.17	43.2	1.44	54.0	1.38	30.3	1.24	39.3
Bintje	57.5	1.42	38.0	1.34	30.0	1.37	30.0	1.31	47.8	1.39	40.7
Elsa	65.0	1.46	46.0	1.38	41.3	1.46	45.5	1.36	32.5	1.30	46.1
Sv 70116	100.0	1.57	66.5	1.46	58.7	1.54	64.5	1.44	61.5	1.44	70.2
LSD ($P < 0.05$)		0.14		0.11		0.12		0.18		0.17	
Percentage error[3]		9.0		9.2		7.3		12.5		12.8	
Sources of variation (F value)[4]											
Cultivar[5] (C)		8.5***		23.9***		6.5***		2.1 NS		2.9*	
Leaf position[6] (LP)		1.6 NS		10.8***		2.1 NS		3.5*		3.8 NS	
Replication[7]		4.7*		1.8 NS		0.1 NS		0.1 NS		0.1 NS	
C × LP		0.4 NS		5.2**		1.4 NS		0.4 NS		0.2 NS	
Dosage[8], zoospores/cm²		200		200		300		200		200	

* See Table 2 - *Siehe Tabelle 2* - *Voir le tableau 2*

[1] *Sorte* - *Variété*; [2] *Mittel* - *Moyenne*; [3] *Fehlerprozent* - *Coefficient de variation*; [4] *Variationsursachen (F-Wert)* - *Source de variation (valeur de F)*; [5] *Sorte* - *Variété*; [6] *Plazierung des Blattes* - *Position de la feuille*; [7] *Wiederholung* - *Répétition*; [8] *Dosierung* - *Dosage*.

Tabelle 3. IE^{1000} von fünf auf der Blattunterseite inokulierten Sorten, 1971.
Tableau 3. IE^{1000} de 5 variétés inoculées sur la surface de la feuille la plus basse, 1971.

Table 4. IE^{1000} of nine cultivars inoculated on the lower leaf surface, 1972.

Cultivar[1]	27 July		3 August		10 August		17 August		24 August		Mean[2]
	A*	B*	A	B	A	B	A	B	A	B	
SvUL 72156	6.4	1.20	21.2	1.30	6.9	1.07	11.0	1.30	11.0	1.14	11.3
Sv 69113	14.6	1.35	11.6	1.18	7.6	1.10	25.0	1.46	51.0	1.38	22.0
71/2023	30.2	1.50	24.8	1.33	19.3	1.20	18.2	1.39	25.5	1.29	23.6
Sv 68112	31.2	1.51	40.4	1.42	9.0	1.11	—	—	17.0	1.22	24.4
Elsa	38.8	1.56	43.6	1.43	25.5	1.24	18.2	1.39	22.5	1.27	29.7
SvUL 71141	34.4	1.53	44.0	1.43	17.9	1.18	—	—	36.0	1.34	33.1
Sv 64110	40.8	1.57	54.0	1.47	22.8	1.22	18.2	1.40	57.0	1.44	38.6
Sv 70116	34.8	1.53	53.2	1.46	35.2	1.27	—	—	51.0	1.41	43.6
Provita	41.4	1.56	46.8	1.44	44.9	1.32	—	—	57.5	1.42	47.7
LSD ($P < 0.05$)		0.07		0.07		0.10		0.06		0.09	
Percentage error[3]		5.5		5.7		9.2		5.0		7.8	
Sources of variation (F value)[4]											
Cultivar[5] (C)		61.4***		41.8***		17.2***		12.9***		19.6***	
Leaf position[6] (LP)		7.0*		3.9*		5.3**		1.0 NS		12.5***	
C × LP		2.5***		2.1**		2.8***		3.0 NS		2.7**	
Dosage[8], zoospores/cm²		500		250		145		510		200	

* See Table 2 - *Siehe Tabelle 2 - Voir le tableau 2*

[1-8] *Siehe Tabelle 3 - Voir le tableau 3*

Tabelle 4. IE^{1000} von neun auf der Blattunterseite inokulierten Sorten, 1972.
Tableau 4. IE^{1000} de neuf variétés inoculées sur la surface de la feuille la plus basse, 1972.

Table 5. IE^{1000} of two cultivars at two levels of fertilizer application, 1972.

Cultivar[1]	NPK 8-15-25	17 July		1 August		15 August		Mean[2]	Field assessment[3]	
	(kg/ha)	A*	B*	A	B	A	B	A	% defo-[4] liation	% blighted[5] tubers
Bintje	400	16.5	1.15	30.8	1.34	5.5	1.24	17.6	7.2	4.2
Grata	400	25.2	1.23	35.2	1.37	9.5	1.31	23.3	1.1	0.5
Bintje	1200	32.0	1.29	34.8	1.37	11.0	1.34	25.9	11.1	10.2
Grata	1200	21.5	1.20	24.6	1.31	3.8	1.16	16.6	3.1	0.6
Mean Bintje	—		1.22		1.36		1.29	21.8		
Mean Grata	—		1.22		1.34		1.24	20.0		
Mean Grata	400		1.19		1.36		1.28	20.5		
Mean Grata	1200		1.25		1.34		1.25	21.3		
LSD ($P < 0.05$)			0.08		0.06		0.07			
Percentage error[6]			7.1		4.7		6.2			
Sources of variation (F value)[7]										
Treatment[8]			8.2***		5.6**		21.7***			
Leaf position[9]			3.2 NS		1.7 NS		8.6**			
Dosage[10], zoospores/cm^2			135		225		600			

* See table 2 - *Siehe Tabelle 2 - Voir le tableau 2*

[1] *Sorte - Variété;* [2] *Mittel - Moyenne;* [3] *Feldbeurteilung - Evaluation au champ;* [4] *Blattfall - Destruction de feuilles;* [5] *Von Knollenfäule befallene Knollen - Tubercules malades;* [6] *Fehlerprozent - Coefficient de variation;* [7] *Schwankungsursachen (F-Wert) - Source de variation (valeur de F);* [8] *Behandlung - Traitement;* [9] *Plazierung des Blattes - Position de la feuille;* [10] *Dosierung - Dosage.*

Tabelle 5. IE^{1000} von zwei Sorten bei zwei Stufen der Düngeranwendung, 1972.
Tableau 5. IE^{1000} de deux variétés à deux niveaux de fumure, 1972.

Table 6. Lesion diameter (mm) on detached leaves of ten cultivars incubated four days, 1972.

Cultivar[1]	20 July				27 July				3 August				10 August				24 August			Mean[2]
	t*	i*	b*	MV	t	i	b	MV	t	i	b	MV	t	i	b	MV	t	i	MV	
SvUL 72156	4	7	11	7.3	8	10	11	9.4	7	6	16	9.5	7	8	8	7.4	11	12	11.4	9.0
Provita	15	15	—	(15.3)	—	—	—	—	8	11	11	9.8	—	—	—	—	11	14	12.3	11.1
Sv 69113	12	14	17	14.3	9	15	16	13.3	8	10	9	9.2	10	11	15	11.8	7	11	9.0	11.5
71/2023	8	11	16	11.7	16	18	19	17.7	13	16	19	15.8	11	10	13	11.0	17	16	16.4	14.5
Elsa	11	13	—	(12.3)	17	20	30	22.3	9	14	11	11.2	11	14	18	14.3	11	13	12.0	15.0
Sv 68112	11	12	18	13.6	23	28	29	26.6	9	10	14	10.8	9	12	14	11.3	15	17	15.8	15.6
Sv 70116	9	20	—	(14.5)	14	22	28	21.3	12	12	21	14.9	17	14	17	16.1	9	15	17.1	17.4
Sv 64110	13	19	19	17.0	25	34	32	30.6	14	15	19	16.1	17	16	18	16.8	21	17	18.9	19.9
SvUL 71141	11	17	24	17.5	30	27	31	29.4	23	21	22	22.2	13	15	15	14.1	15	21	18.3	20.3
Bintje	15	24	24	21.0	24	35	35	31.1	14	18	16	15.8	13	21	18	17.2	—	—	—	21.3
Mean				14.6				22.4				13.5				13.3			14.6	
LSD ($P < 0.05$)				3.8				5.4				3.2				2.5			3.0	
Percentage error[3]				29.4				27.3				26.7				25.9			28.8	
Source of variation (F value)[4]																				
Cultivar[5] (C)				31.8***				48.4***				39.8***				25.9***			13.6***	
Leaf position[6] (LP)				55.9***				33.9***				33.5***				16.7***			3.6 NS	
C × LP				1.9*				2.6**				4.8***				2.7***			3.4**	

* Leaf position: t = top, i = intermediate, b = bottom - *Plazierung des Blattes: t = oben (Wipfel), i = Mitte; b = unten* - *Position de la feuille: t = cime, i = intermédiaire, b = pied*

1–6 *Siehe Tabelle 3* - *Voir le tableau 3*

Tabelle 6. Läsionsdurchmesser (mm) auf gepflückten Blättern von zehn Sorten nach vier Tagen Inkubation, 1972.
Tableau 6. Diamètre des lésions (mm) sur feuilles détachées, de 10 variétés, après un passage de 4 jours à l'incubateur, 1972.

Table 7. Conidia/lesion (leaflet) × 10^3 on detached leaves after drop-inoculation. Incubation 5 days, eleven cultivars.

Cultivar[1]	1971					1972								
	20 July			25 August	mean[2]	20 July	27 July		3 August		10 August		24 August	mean
	t*	i*	b*	t		t	t	i	t	i	t	i	t	
SvUL 72156					—	15.9	15.9	17.0	13.5	16.7	14.0	13.6	15.7	15.28
SvUL 71141					—	15.9	20.8	20.6	19.6	21.0	13.2	14.3	19.0	18.04
Elsa	18.8	30.7	25.2	12.6	21.83	17.2	18.5	20.7	18.9	19.2	15.7	15.1	21.3	18.33
Sv 69113	9.1	4.3	—	14.5	(10.01)	18.7	17.8	22.1	15.4	20.3	19.1	17.3	20.1	18.61
Sv 68112					—	18.3	18.3	19.9	15.6	24.3	15.2	17.6	20.5	18.71
Prominent	12.5	18.6	34.2	4.4	17.41									—
Provita					—	17.8	18.8	19.8	18.7	20.4	—	—	16.8	(18.72)
71/2023					—	17.6	19.4	20.0	19.1	19.3	17.1	16.9	22.5	18.99
Sv 64110					—	18.1	21.2	20.2	17.3	20.2	15.7	19.6	21.9	19.28
Sv 70116	7.0	15.3	35.2	22.9	20.08	19.1	21.4	22.9	20.2	24.4	18.1	16.8	21.1	20.49
Bintje	37.4	30.8	30.4	9.4	27.02	18.2	21.7	24.7	25.0	23.7	19.0	21.5	—	(21.99)
LSD ($P < 0.05$)						2.5	1.9	0.4	1.1	2.6	2.0	0.7	1.8	
Percentage error[3]						8	9	3	12	9	8	4	5	
Source of variation (F value)[4]														
Cultivar[5]						1.2 NS	3.2*	30.1***	3.3*	5.9***	3.8*	21.5***	7.7**	
Replication[6]						0.0 NS	8.3**	0.2 NS	0.8 NS	2.7 NS	0.0 NS	0.4 NS	0.7 NS	

* See Table 6 - Siehe Tabelle 6 - Voir le tableau 6

[1-5] *Siehe Tabelle 3 - Voir le tableau 3*; [6] *Wiederholung - Répétition*

Tabelle 7. Konidie/Läsion (Blättchen) × 10^3 auf gepflückten Blättern nach Tropfeninokulation. Inkubation 5 Tage, elf Sorten.

Tableau 7. Conide/lésion (foliole) × 10^3 sur feuilles détachées après inoculation par goutte. Passage de 5 jours à l'incubateur, 11 variétés.

(about 70 days from planting) followed by a rise again in IE during the second half of August. In two cultivars, Sv 69113 and Sv 64110, this rise at the approach of senescence was pronounced in 1972.

Varietal differences in lesion size (LES)
Varietal differences in lesion size after four days of incubation were highly significant (Table 6). A maximum in average lesion size was reached at the end of July. Leaves from the lower portions of the plant were in general more susceptible to mycelial growth. Only occasionally was there such a differential effect between top and intermediate leaves. The average coefficient of variation was 27.6 %, but ten measurements per treatment (cultivar and leaf position) have been sufficient to detect statistically significant differences.

Variental differences in number of conidia (CON)
The number of conidia per lesion varied in the 1972 test from 13 200 to 25 000 (Table 7). Varietal differences were significant at the top leaf position and highly significant at the intermediate position. In general conidia production was higher on leaves at the intermediate position than on leaves at the top position.

Discussion

The quantitative inoculation technique can be a useful tool in studies of the effect of fungicidal treatments e.g. application methods or comparison amongst chemical compounds. The technique is sufficiently precise to detect even small differences, difficult to establish by the subjective scoring methods used in field assessments of disease attack.

The data of Tables 1 and 2 refer to inoculation of the lower leaf surface and thus demonstrate the success of infection on the more susceptible lower leaf surface. This is also the part of the leaf most difficult to cover with sufficient amounts of fungicides and therefore the most critical part of the crop canopy in relation to treatment. A detailed analysis as given in Tables 1 and 2 gives more information on coverage and redistribution of the fungicide than field assessments.

The proposed component analysis of field resistance with measurement of the three parameters IE, LES and CON gives more information to the breeder than normal field assessments of per cent defoliation. It adds to a better understanding of field resistance and gives a means of defining the aim of the breeding efforts. The effect of each component can be measured in relation to the over all slowing down of the disease. Selection can thus be made for a specific character and studies of inheritance can be made with higher accuracy. Studies of a possible shift towards a greater capacity of the late blight fungus to overcome field resistance (Anonymous, 1973) can also be monitored by using a standard quantitative technique, such as the one described here.

Data of the above parameters can be fed into a simulator programme in order to

Table 8. A mathematical model to quantify leaf destruction, due to late blight, from three parameters of field resistance.

Number of infections[1]	$x_n = (x_{n-1}\,(CON \cdot 10^{-2})\,(IE_v)$
Destroyed leaf area[2]	$y_n = (x_n\,(LES)$
Example: Cultivar Bintje[3]	$IE_V = 0.0450$ infections[4]
	$LES = 6.93\ cm^2$
	CON = 22 000 conidia/lesion
	n = number of spore generations[5]
	Initial inoculum[6] = 100 000 spores
	Spore survival[7] = 1:100

Spore generation	Number of infections	Destroyed leaf area in cm^2	
		per generation	accumulated
1	45.0	311.9	311.9
2	445.5	3 087.3	3 399.2
3	4 410.5	30 564.8	33 964.0
4	43 664.0	302 591.5	336 555.5
5	432 273.6	2 995 656.0	3 332 211.5

[1] *Anzahl Infektionen - Nombre de contaminations;* [2] *Zerstörte Blattfläche - Surface de feuille détruite;* [3] *Beispiel: Sorte Bintje - Exemple: variété Bintje;* [4] *Infektionen - Contaminations;* [5] *Anzahl Sporengenerationen - Nombre de générations de spores;* [6] *Anfangsinokulum - Inoculum initial;* [7] *Ueberleben der Sporen - Survie des spores;* [8] *Sporengeneration - Générations de spores;* [9] *Anzahl Infektionen - Nombre de contaminations;* [10] *Pro generation - Par génération;* [11] *Akkumuliert - Cumulée*

Tabelle 8. Ein Rechenmodell zur Bestimmung der durch Krautfäule verursachten Blattzerstörung aus drei Feldresistenzparametern.
Tableau 8. Un modèle mathématique pour quantifier la destruction de feuilles par le mildiou, à l'aide de 3 paramètres de résistance au champ.

study varietal differences of disease progress under different climatic conditions. Complications such as lack of 'blight weather', lack of compatible races, and inter-plot interference are ruled out.

A very simple methematical model (Table 8) illustrates disease progress during five *Phytophthora* generations on cv. Bintje. The number of infections at any generation (x_n) is a function of the number of infections in the previous generation, $x_{(n-1)}$, the number of conidia produced per infection (CON) and IE. The leaf area destroyed at the end of the same generation (y_n) is a function of the number of lesions (x_n) and the size of each lesion (LES).

Data of IE, LES and CON for seven cultivars included in the present investigation have been used to calculate the leaf area destroyed after five generations of blight in order to illustrate the range of varietal differences in disease progress (Table 9). A ten-thousand-fold difference is demonstrated for the two extremes.

The ranking order follows closely field assessments made in the Toluca Valley (Mexico) and Svalöv (Sweden).

Table 9. Component analysis of field resistance and field assessments in Mexico and Sweden.

Clone[1]	IE_v^{1000}	LES	CON × 1000	Destroyed leaf area in cm^2 after 5 generations[2]	Field assessment[3]	
					Mexico*	Sweden**
SvUL 72156	11.3	1.14	15	241	3	0.12
Sv 69113	22.0	2.53	19	22 330	3	0.10
Sv 71/2023	23.6	2.95	19	36 195	3+	0.20
Sv 68112	24.4	3.87	19	55 578	4+	0.20
Provita	47.7	2.38	19	860 879	-	0.23
Sv 70116	43.6	4.33	20	1 232 918	5	0.29
Alpha	-	-	-	-	5	0.30
Bintje	45.0	6.93	22	3 332 212	-	0.36

* Scoring 1-5 when Alpha is 5 (dead - *Bonitierung von 1-5, wenn Alpha 5)tot)ist - Notation 1-5, quand Alpha est 5 (détruite)*

** Infection rate r = the regression coefficient of loge [x/(1-x)] on time according to van der Plank (1963) derived from Fig. 5. Estimates averaged for the entire period of the graphs except for Sv 70116, where r is given for the logarithmic phase of the graph (see text) - *Infektionsrate r = Regressionskoeffizient von loge [x/(1-x)] (Zeit) gemäss van der Plank (1963), abgeleitet aus Abb. 5 . Durchschnittsschätzungen über den ganzen Ruwenverlauf, ausgenommen für Sv 70116, wo r die logarithmische Phase der Kurve angegeben ist (siehe Text) - Taux d'infection r = coefficient de régression de loge [x/(1-x)]selon Van der Plank (1963), dérivé de la fig. 5. Evaluations moyennes pour la période à l'exception de Sv 70116, pour lequel r est donnë pour la forme logarithmique de la courbe (voir le texte).*

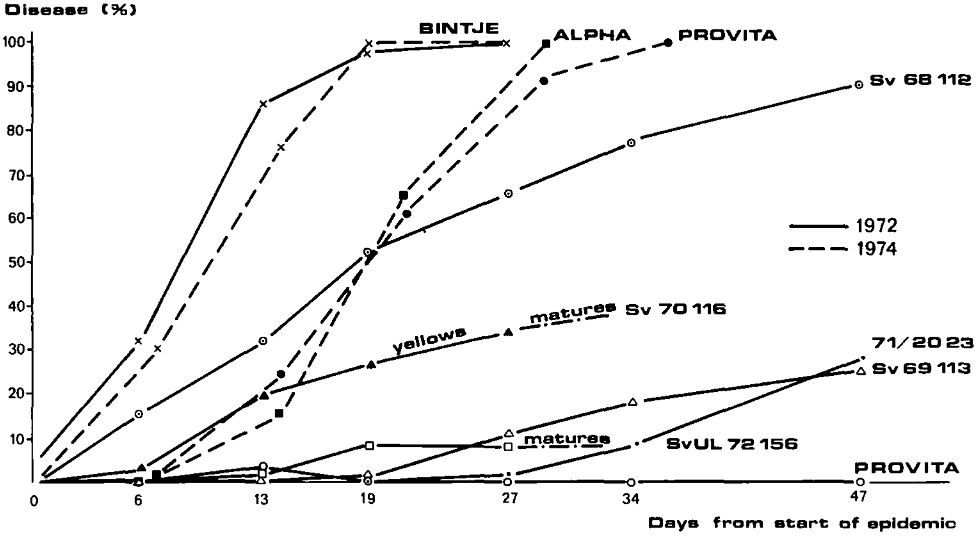
[1] *Klon - Clone;* [2] *Zerstörte Blattfläche in cm^2 nach 5 Generationen - Surface de feuille détruite en cm^2 après 5 générations;* [3] *Feldbeurteilung - Evaluation au champ.*

Tabelle 9. Analyse der Komponenten der Feldresistenz und Feldbeurteilungen in Mexiko und Schweden.
Tableau 9. Composants de l'analyse de la résistance au champ et de l'évaluation au champ au Mexique et en Suède.

Fig. 4 illustrates the range in rate of disease progress in the clones used in the present investigation. It also illustrates the misleading information sometimes collected from field observations. Cultivar Provita is an *R* genotype, which in the 1972 test escaped infection due to lack of a compatible race of *P. infestans* in the spontaneous disease epidemic of that year. In 1974 the test plots were artificially inoculated with race 1.2.3.4.5.7 25 days prior to the start of the epidemic and this race succeeded in spreading through the field securing good readings of fields resistance, including those on Provita and other *R* genotypes.

Clone Sv 70116 was approaching maturity at the time of the epidemic, and the infection rate did not follow the pattern to be expected from the logarithmic phase of the curve. The Toluca readings were taken about one month earlier, and there the clone was similar to Alpha in degree of attack.

Fig. 4. Field assessment of blight attack in Svälov 1972 and 1974.



Disease - *Krankheitsbefall - Maladie*
Days from start of epidemic - *Tage seit dem Beginn des epidemischen Auftretens - Jours après le début de l'épidémie*
Yellows - *Gelbe - Jaunes*
Matures - *Reife - Mûrs*

Abb. 4. Feldbeurteilung von Krautfäulebefall in Svälov 1972 und 1974.
Fig. 4. Evaluation au champ d'une attaqua de mildiou à Svälov en 1972 et 1974.

Acknowledgments

The assistance of hortonom Ulla Böös and fil.lic. Lennart Erjefält is appreciated. The authors also wish to thank the Swedish Council for Forestry and Agricultural Research for financial support and the International Potato Center, Lima, Peru, through which this work was completed within the framework of a linkage program.

Zusammenfassung

Eine Labormethode zur Messung des Krankheitsbefalls, verursacht durch Phytophthora infestans

Es wird eine Methode beschrieben, die auf quantitativen Messungen des Infektionserfolges (IE), des Läsionenwachstums (LES) und der Konidienproduktion (CON) beruht. Um die Methode zu testen, wurden Blattmuster aus Feldversuchen genommen, die zum Studium von Fungizidbehandlungen (Tabellen 1 und 2) und der Resistenz von Kartoffelsorten gegen Krautfäule (Tabellen 3-7) angelegt wurden.

Die Methode ist genügend genau, um selbst kleine Unterschiede zu erfassen, die mit den bei Feldbeurteilungen angewendeten subjektiven Bewertungsmethoden nach Punkten schwerlich festgestellt werden. Die Daten der drei Parameter wurden zur Errechnung der durch Krautfäule zerstörten Blattfläche verwendet, um die sortenbedingten Unterschiede im Krankheitsverlauf zu illustrieren und die Ergebnisse mit den Feldbeurteilungen zu korrelieren (Tabellen 8, 9 und Abb. 4).

Résumé

Une méthode de laboratoire pour la mesure des attaques de Phytophthora infestans

La méthode décrite est basée sur une mesure quantitative du taux de contamination (IE = infection efficiency), des lésions en végétation (LES) et de la production de conidies (CON).

Pour tester la méthode, des échantillons de feuilles ont été prélevés dans les parcelles d'essais mis en place pour l'étude de fongicides (tableaux 1 et 2) et pour l'étude de la résistance au mildiou de diverses variétés (tableaux 3-7).

La technique est suffisamment précise pour détecter de faibles différences, difficiles à établir par une méthode de notation subjective utilisée pour les évaluations au champ.

Les données de trois paramètres ont été utilisées pour calculer la surface foliaire détruite par le mildiou, afin de mettre en évidence des différences variétales dans la progression de la maladie et de mettre en corrélation les résultats et l'évaluation au champ (tableaux 8 et 9, fig. 4).

References

Anonymous, 1973. Late blight strategy. Report of the International Potato Center's late blight project planning conference. CIP, La Molina, Apartado 5969, Lima, Peru.

Björling, K., & K. A. Sellgren, 1955. Deposits of sporangia and incidence of infection by *Phytophthora infestans* on upper and lower surfaces of potato leaves. *Acta Agric. scand.* 5: 375-386.

Carlsson, B., 1969. Electronic particle counting. A new method for counting eggs and larvae of *Heterodera* species. *Stat. Växtsk. Anst. Medd.* 14:126.

James, W. C., C.S. Shih, L. C. Callbeck & W. A. Hodgson, 1973. Interplot interference in field experiments with late blight of potato (*Phytophthora infestans*). *Phytopathology* 63: 1269-1275.

Kleczkowski, A., 1949. The transformation of local lesion counts for statistical analysis. *Ann. appl. Biol.* 36: 139-152.

Plank, J. E. van der, 1963. Plant diseases: Epidemics and control. Academic Press, New York and London.

Schein, R. D., 1964. Design, performance, and use of a quantitative inoculator. *Phytopathology* 54: 509-513

Umaerus, V., 1969a. Studies on field resistance to *Phytophthora infestans*. 1. The infection efficiency of zoospores of *P. infestans* as influenced by the host genotype. *Z. PflZücht.* 61: 29-45.

Umaerus, V., 1969b. Studies on field resistance to *Phytophthora infestans*. 2. A method of screening young potato seedlings for field resistance to *P. infestans*. *Z. PflZücht*, 61: 167-194.