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# Photosynthesis is not impaired in healthy tissue of blighted potato plants

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**Abstract**

The net photosynthetic rates of green leaf tissue of potato plants of different cultivars were measured in the field and in a controlled environment after infection of the plants by *Phytophthora infestans*.

Infection had no significant effect on the net photosynthetic rate at light saturation, the efficiency of light use at low light intensities, or dark respiration. The reported effect of *P. infestans* on tuber yield seems to be caused solely by a reduction in the green leaf area. Therefore, a high rate of photosynthesis in green leaf tissue of infected plants is not a good selection criterion for potato genotypes.

*Additional keywords:* late blight, *Phytophthora infestans*, dark respiration.

## Introduction

The loss in tuber yield of potatoes infected by late blight, caused by *Phytophthora infestans* (Mont.) de Bary, varies with the host cultivar and the growing environment. Haverkort and Bicamumpaka (1986), and Waggoner and Berger (1987) have recently shown that these differences in yield loss can largely be explained by differences in cumulative light interception by green leaf tissue. There does not seem to be an effect on the radiation use efficiency (the ratio of tuber yield and cumulative light interception). Infection by the fungus reduces the green leaf area, by lesion growth and by stimulation of chlorosis and necrosis, but the photosynthetic activity of the remaining green leaf tissue is apparently not impaired. However, the constancy of the radiation use efficiency does not give conclusive evidence for this hypothesis, because it is a rough measure of crop productivity as it includes seasonal variations in light interception, leaf photosynthesis, respiration and assimilate partitioning. Only direct measurements of photosynthetic rates in healthy and blighted plants can show whether the photosynthetic activity of the green leaf area is affected by late blight. Measurements of photosynthetic rates could be used to screen for host genotypic differences and thus help in the selection of blight tolerant genotypes for breeding purposes.

There are an increasing number of reports on the direct measurement of photosynthetic rates in diseased plants. Farrar and Lewis (1987) gave examples of both positive and negative systemic effects of fungal infection on leaf photosynthetic rates. The effect of a fungus on host photosynthesis depends on the pathosystem in question. Scharen and Krupinsky (1969), and Berghaus and Reisener (1985) reported that variability bet-

ween host genotypes may also exist. They found that photosynthetic rates were reduced to different extents after infection of wheat cultivars by *Septoria nodorum* and *Puccinia graminis*, respectively. So far, no reports on the effect of *P. infestans* on the rates of photosynthesis in potatoes have been published.

We measured, under controlled conditions, the rate of photosynthesis at light saturation ($P_m$), the light use efficiency at low light intensities ($\epsilon$) and the dark respiration ($R_d$) of green leaves of healthy and partly blighted potato plants of two cultivars. We also measured, in the field, the $P_m$ of three different potato cultivars infected to various degrees by blight.

## Materials and methods

Plants grown outdoors in pots were inoculated in a greenhouse five weeks after planting, and were placed in a climate chamber two weeks later to measure photosynthesis-light response curves. In a second experiment, the inoculation and measurement of light-saturated rates of photosynthesis were carried out in the field.

*Pot experiment.* Individual seed tubers of the mid-early potato cultivar Bintje and the mid-late cultivar Surprise were planted in pots containing 10 l of peat soil on July 20, 1988. Stems emerged during the first week after planting, and these were trimmed to one per plant within 14 days. For both cultivars 35 pots were placed in the open air and thus subjected to natural weather conditions. All pots were sprayed weekly until 30 days after planting with the mild contact fungicide chlorothalonil to prevent late blight infection while minimizing phytotoxic or other effects on the plants. The plants were transferred into the greenhouse 35 days after planting. Inoculum was prepared by making a suspension of sporangia washed off leaves of cv. Bintje plants, inoculated one month before with the complex *P. infestans* race 1, 2, 3, 4, 5, 7, 10, 11. Test plants were inoculated 36 days after planting by spraying inoculum (146 000 sporangia $ml^{-1}$; about half the sporangia had germinated and released zoospores) over the lower two-third of leaf layers of the plants. Test and control plants were then capped with plastic bags to increase the humidity around the leaves. This procedure was repeated the next day with a suspension of 43 000 sporangia $ml^{-1}$.

Photosynthesis-light response curves were determined for eight replicates 47 to 50 days after planting. The measurement scheme followed a randomized block design with concurrent measurements of blocks. For this purpose every morning and afternoon four plants were transferred into a climate chamber (20 °C) at the Centre for Agrobiological Research (CABO) in Wageningen. The number of leaves were counted and disease severities were estimated, with the naked eye, for each separate leaf and stem internode. The plants had formed 17 to 18 leaves with distal leaflets longer than 5 cm. $CO_2$-exchange was measured using leaf 14 or 15 counting from the soil level, i.e. on relatively young, un-inoculated leaves (Louwerse and Van Oorschot, 1969). The light intensity was reduced stepwise from about 280 W $m^{-2}$ photosynthetically active radiation (400-700 nm) through four intermediate light levels to complete darkness. The plants were allowed to adapt for more than thirty minutes at every light intensity. Finally, the measured leaves were harvested to determine surface area, dry weight and total nitrogen content.

The photosynthetic data of each plant were fitted by non-linear regression analysis

to a negative exponential function of light intensity (De Wit et al., 1978):

$$P = (P_m + R_d) \times (1 - \exp(- I \times \epsilon/(P_m + R_d))) - R_d \quad (1)$$

where $P$ is the net $CO_2$ assimilation rate (g $m^{-2}$ $h^{-1}$), $P_m$ is the net $CO_2$ assimilation rate at light saturation (g $m^{-2}$ $h^{-1}$), $R_d$ is the dark respiration rate (g $m^{-2}$ $h^{-1}$), $\epsilon$ is the initial light use efficiency (g $J^{-1}$ s $h^{-1}$) and $I$ is the incident photosynthetically active radiation (W $m^{-2}$). The results were analysed with a multifactorial analysis of variance with block, genotype and treatment as independent variables.

*Field experiment.* Plots of the cultivars Bintje and Surprise, and of the late cultivar Pimpernel were laid out at distances of 9 to 14 m on a sandy soil in a sugar beet crop, to minimize interplot interference. Per plot of 4 by 3.75 m, 60 tubers were planted on April 29, 1987. Fifty per cent emergence was reached 20 days after planting for 'Bintje', followed by 'Pimpernel' and 'Surprise' four and five days later. The experiment was arranged in a fully randomized design with three genotypes and three treatments in four replicates. One third of the plots was sprayer-inoculated 55 days after planting with a suspension of *P. infestans* (race 1, 2, 3, 4, 5, 7, 10, 11; 150 ml per plot; 20 000 sporangia $ml^{-1}$. Another third of the plots, the controls, received regular sprayings with contact fungicide (maneb-tin) throughout the growing season, and remained practically free from late blight. The last third was left to natural infection, inoculum or fungicide was not sprayed.

Rates of photosynthesis were measured in different, randomly selected plots on seven days, in July and August. This was carried out with a portable leaf chamber analyzer (LCA; Analytical Development Co. (ADC), UK). All measurements were done at light saturation. An incandescent lamp cooled by a fan was held over the enclosed leaf for at least one minute; the light intensity was 400 W $m^{-2}$ of photosynthetically active radiation. The rate of photosynthesis was calculated following the procedure described by von Caemmerer and Farquhar (1981). The rate of photosynthesis of four distal non-infected leaflets of two or three leaf layers (top third, middle third and – if still present – bottom third) was measured in each selected plot. The four leaflets from a leaf layer were harvested as a group and total dry matter, leaf area and nitrogen content were determined.

Because the experimental design was non-orthogonal, the results were analysed using multiple linear regression on dummy variables (Snedecor and Cochran, 1980, p. 421), with day of measurement, genotype and treatment as independent variables.

## Results

For each plant in the pot experiment, disease severity values were calculated separately for leaves and stems. Disease severity was expressed as a percentage of lesion coverage of leaves or stem *below* the measured leaf. The leaves measured were green, symptomless leaves from the un-inoculated tops of the plants. When photosynthesis was measured, the average disease severity of inoculated plants was between 10 and 20%, with no significant differences between 'Bintje' and 'Surprise' (Table 1). All inoculated plants, except one 'Bintje' plant, had at least one stem lesion that completely encircled the stem at some point below the measured leaf.

Table 1. Gas exchange parameters and plant characteristics of two potato cultivars, pot experiment. Parameters: photosynthetic rate at light saturation ($P_m$), dark respiration ($R_d$), initial light use efficiency ($\epsilon$). Characteristics: disease severity, leaf nitrogen (N) content and specific leaf weight (*SLW*). Means and standard errors of difference.

| Cultivar | Treatment | $n$[1] | $P_m$ | $R_d$ | $\epsilon$ | Lesions | | N-content | *SLW* |
|---|---|---|---|---|---|---|---|---|---|
| | | | ($g\ m^{-2}\ h^{-1}$) | ($g\ m^{-2}\ h^{-1}$) | ($g\ MJ^{-1}$) | leaves (%) | stem (%) | ($g\ N\ m^{-2}$) | ($g\ m^{-2}$) |
| Bintje | inoculated | 8 | 5.66 | 0.30 | 20.4 | 19.8 | 16.2 | 2.89 | 56.2 |
| Bintje | control | 8 | 5.71 | 0.27 | 17.8 | 0.0 | 0.0 | 3.33 | 57.4 |
| Surprise | inoculated | 8 | 4.70 | 0.29 | 18.7 | 16.5 | 13.2 | 2.48 | 44.3 |
| Surprise | control | 8 | 4.99 | 0.27 | 16.8 | 0.0 | 0.0 | 2.64 | 47.6 |
| | S.E.D.[2] | | 0.43 | 0.02 | 1.5 | 4.7 | 3.1 | 0.16 | 3.7 |

[1] Number of replicates. Each replicate represents one photosynthesis-light response curve with observations at six difference light intensities.
[2] Standard error of difference of means. For lesion percentages only calculated for the inoculation treatment.

Infection with *P. infestans* did not significantly affect the photosynthesis parameters of either 'Bintje' or 'Surprise' (Table 1). The rate of photosynthesis at light saturation was higher for 'Bintje' than for 'Surprise' ($P < 0.05$). This may be explained partly by differences in the nitrogen content per unit leaf area or specific leaf weight (SLW) of the cultivars (Table 1). The nitrogen content and specific leaf weight were closely correlated ($r^2 = 0.79$, $n = 32$), and both showed a weak positive correlation with $P_m$ ($r^2 = 0.28$ in both cases, $n = 32$).

In the field experiment, photosynthesis was measured in July (71, 72, 78 days after planting) and August (days 99, 106, 107 and 110). The average conditions of the plants on days 62 and 98, i.e. before these measurements, are shown in Table 2. At 62 days after planting, plants of all cultivars and treatments had formed 11 to 12 leaves per main stem, and had already dropped one or two leaves. Cv. Bintje had almost completed its leaf formation by day 62, whereas subsequent leaf formation was more pronounced in the late cultivar Pimpernel than in Surprise. Inoculation significantly reduced the number of leaves in these two cultivars.

Table 2 also gives data on stem lesion coverage and on leaf lesion coverage in the three canopy layers where the photosynthesis measurements were taken. As expected, disease development was strongest in inoculated plots, followed by the unsprayed plots, while the controls remained free of disease. Cultivar Bintje was more severely diseased than cvs Surprise and Pimpernel. Leaf lesions developed fastest in the lower layers of the canopy.

The results of the photosynthesis measurements are given in Fig. 1. $P_m$ varied with leaf position, being highest in the top leaf layer. Within leaf layers, $P_m$ showed weak positive correlations with the percentage lesion coverage (from top to bottom: $r^2 = 0.07$, $r^2 = 0.06$, $r^2 = 0.31$). However, treatment effects were only significant in the lower two leaf layers, while significant genotype effects occurred at all levels. The cultivars

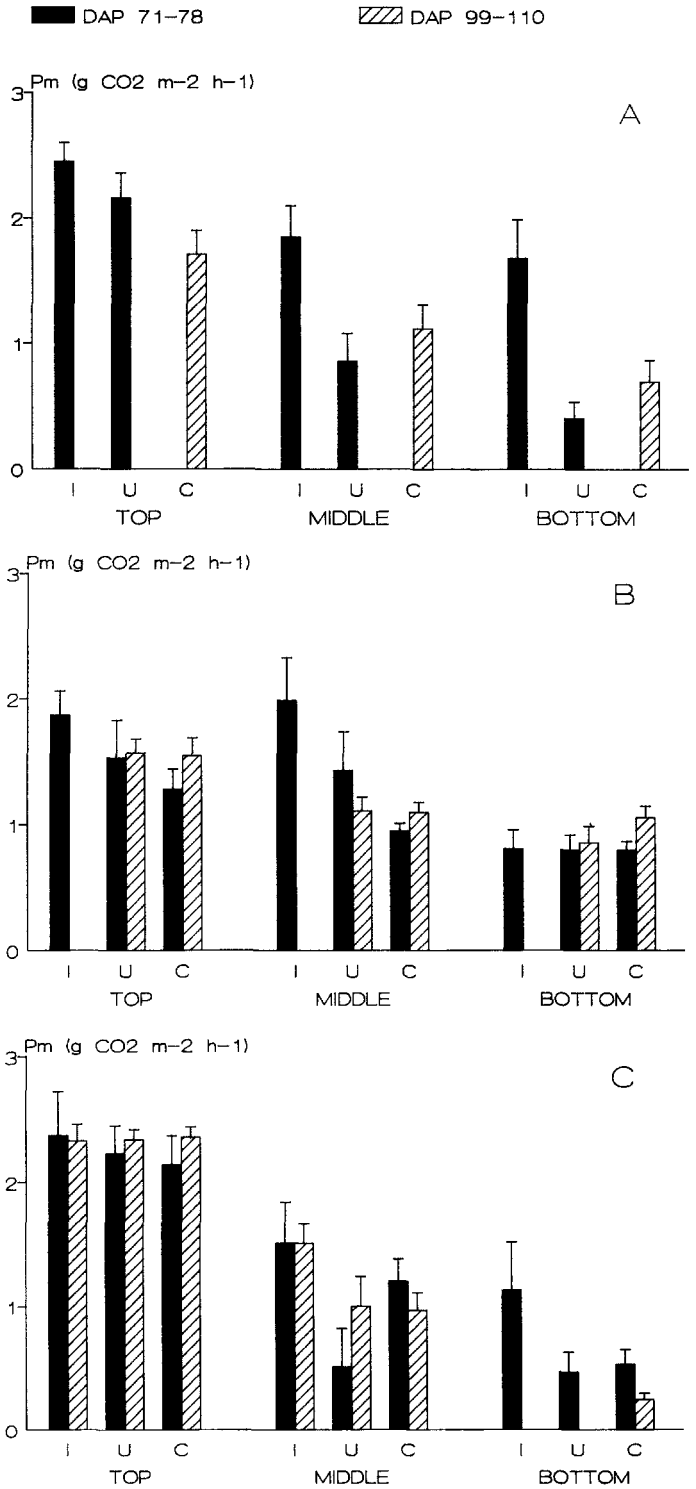
Table 2. Plant characteristics of three potato cultivars, field experiment. Characteristics: percentage leaf lesion coverage at three levels in the canopy, percentage stem lesion coverage, total number of leaves with distal leaflets longer than 5 cm and number of leaves still attached and at least partly green. Data on two days after planting (*DAP*). Standard errors of lesion coverage percentages and leaf numbers were lower than 13.0% and 0.72 respectively, unless otherwise indicated.

| *DAP* | Cultivar | Treatment | Lesions | | | | Leaf number | |
|---|---|---|---|---|---|---|---|---|
| | | | top (%) | middle (%) | bottom (%) | stem (%) | total | green |
| 62 | Bintje | inoculated | 19.4 | 30.6 | 57.8 | 11.4 | 12.4 | |
| | | unsprayed | 0.2 | 2.4 | 0.3 | 0.0 | 12.6 | |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 12.3 | |
| | Surprise | inoculated | 1.3 | 1.2 | 1.1 | 0.1 | 12.1 | |
| | | unsprayed | 0.0 | 0.0 | 0.0 | 0.6 | 13.3 | |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 11.9 | |
| | Pimpernel | inoculated | 2.0 | 6.5 | 9.8 | 0.0 | 11.1 | |
| | | unsprayed | 0.0 | 0.1 | 0.0 | 0.0 | 11.8 | |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 12.0 | |
| 98 | Bintje | inoculated | 100.0 | 100.0 | 100.0 | 100.0 | | 0.0 |
| | | unsprayed | 89.1 | 96.3 | 99.2 | 77.1 | 14.0 | 0.4 |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 14.2 | 9.6 |
| | Surprise | inoculated | 45.3[1] | 60.5 | 87.4 | 60.4 | 13.7 | 1.0 |
| | | unsprayed | 0.0 | 0.6 | 3.2 | 8.1 | 18.0 | 11.0 |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 16.1 | 8.9 |
| | Pimpernel | inoculated | 48.5 | 79.2 | 78.6 | 39.6 | 15.4[2] | 1.0 |
| | | unsprayed | 7.3 | 13.8 | 40.4 | 0.1 | 18.5 | 7.4 |
| | | control | 0.0 | 0.0 | 0.0 | 0.0 | 19.8 | 11.1 |

[1] Standard error is 22.0%.
[2] Standard error is 1.06.

with the lowest $P_m$ values in the top and middle layers were 'Surprise' ($P < 0.01$) and 'Pimpernel' ($P < 0.05$), respectively; a genotype-treatment interaction was not present in these two layers. In the middle leaf layer, the $P_m$ values of inoculated plants were significantly higher than those of unsprayed plants and controls ($P < 0.01$). In the lowest leaf layer, a genotype-treatment interaction was present ($P < 0.01$). The $P_m$ values of inoculated plants of cvs. Bintje and Pimpernel were higher than those of the controls ($P < 0.01$ and $P < 0.05$, respectively), whereas cv. Surprise did not respond to treatment ($P > 0.1$). Thus 'Bintje' had the highest $P_m$ of the inoculated cultivars ($P < 0.01$), while 'Surprise' was superior to 'Pimpernel' in the controls ($P < 0.01$).

The observed differences in $P_m$ between leaf layers and between cultivars were associated with differences in nitrogen content (Fig. 2). The nitrogen content increased with the higher leaf positions, and was generally greatest in the cultivar Pimpernel, followed by cv. Bintje. The nitrogen content also varied with time, showing a decrease


DAP 71–78
DAP 99–110
Pm (g CO2 m–2 h–1)
A
B
C
0
1
2
3
I U C I U C I U C
TOP MIDDLE BOTTOM

◀ Fig. 1. Net photosynthetic rate at light saturation ($P_m$) of three potato cultivars, after three treatments (I = inoculated, U = unsprayed, C = control), measured at three levels in the canopy (top, middle and bottom leaf layer) during two periods (71-78 and 99-110 days after planting (DAP)). Means and standard errors of the mean.
A: cv. Bintje; B: cv. Surprise; C: cv. Pimpernel.

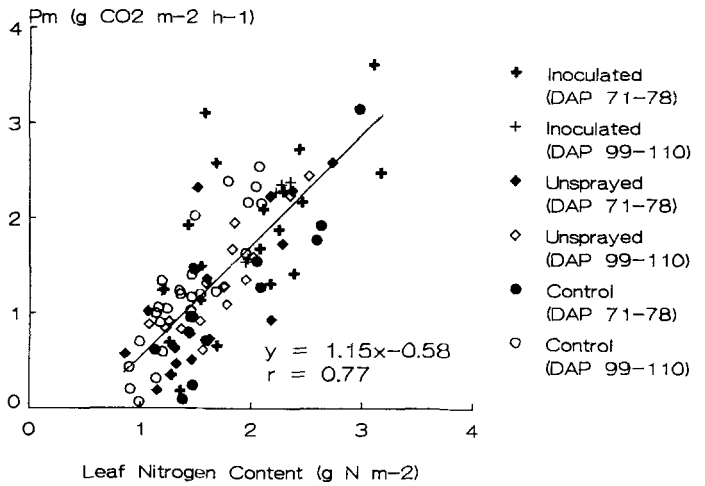



Fig. 2. Relationship between net photosynthetic rate at light saturation and leaf nitrogen content of potato cultivars Bintje, Surprise and Pimpernel. Different symbols indicate different treatments (inoculated, unsprayed or control) and different periods of measurement (71-78 or 99-110 days after planting). Points represent mean photosynthetic rates of four replicates of specific combinations of cultivar, treatment, day of measurement and leaf position.

in 'Surprise' and 'Pimpernel'. The positive correlation between $P_m$ and nitrogen content in the field experiment, when all measurements are taken into consideration, was stronger than in the pot experiment ($r^2 = 0.59$, top layer only: $r^2 = 0.53$), and the correlation of $P_m$ with specific leaf weight was again equally strong (not shown, $r^2 = 0.54$).

## Discussion

The coefficient of variation of individual measurements of $P_m$, calculated as the square root of the error mean square divided by the overall mean, was 17% in the pot experiment and 31% for the top layer measurements in the field experiment. Thus there was a large variation in the pot experiment in spite of the homogeneity of the environmental conditions and the precision of the measurements. This indicates that much of the variation in $P_m$ was due to the intrinsic variation between leaves. Therefore, the number of replicates needed to determine differences in photosynthetic rates between treatments or genotypes is high irrespective of the experimental conditions.

The average $P_m$ value of young leaves was 5.3 g $m^{-2}$ $h^{-1}$ in the pot experiment and 2.0 g $m^{-2}$ $h^{-1}$ in the field. The field values are comparable to those reported by Dwelle (1985) and Firman and Allen (1988) for field-grown potatoes. The high $P_m$ values reported here for the plants of the pot experiment are similar to those found by J. Schans (pers. comm., 1989) for the cultivars Darwina and Irene, grown in pots in the greenhouse and examined with the same equipment used in the present study. Vos and Oyarzun (1987) also used the same equipment, but found $P_m$ values up to 4.0 g $m^{-2}$ $h^{-1}$ for cv. Bintje. They reported a close relationship between age-dependent reduction in leaf nitrogen content and reduction in $P_m$. The present results cannot explain the differences between the $P_m$ of field- and pot-grown plants on the basis of differences in leaf nitrogen content, since the nitrogen content was determined for whole leaves in the pot experiment and only for distal leaflets in the field. Differences between the distribution of lesions over leaf layers probably did not contribute to the discrepancy in rates of photosynthesis. The method of inoculation used in the pot experiment led to healthy plant tops and diseased lower plant parts, as was also seen in plants infected in the field (Table 2). Irrespective of a possible involvement of the leaf nitrogen content or disease pattern, the different growing and measurement conditions may have caused the differences in $P_m$ found in the present study. The pot plants were optimally supplied with water and nutrients, and all leaves continuously received ample light because the pots were widely spaced. The measurement temperature of 20 °C and the longer time of adaptation to high light intensity (30 min in the pot experiment vs. 1 min in the field) could also have increased the $P_m$ in the climate chamber.

A small positive effect on $P_m$ was found in the lower leaf layers of cvs. Bintje and Pimpernel after inoculation, possibly due to reduced shading because of foliage loss. This small effect would have negligible consequences for production. In healthy crops incident light is primarily absorbed by the higher leaf layers. This effect is enhanced in blighted potato plants, where disease occurs mainly in the lower plant parts. In the three potato cultivars studied, infection by *P. infestans* did not have a systemic effect on the $P_m$, $R_d$ and $\epsilon$ of green leaf tissue in the plant tops. Even lesions encircling the stem did not reduce the rate of photosynthesis. Thus vascular transport was not hampered, and nor were toxic substances secreted. This is consistent with the insensitivity of the crop radiation use efficiency to the disease, as has been reported in the literature for a wide range of potato genotypes (Haverkort and Bicamumpaka, 1986; Waggoner and Berger, 1987).

It can be concluded that the photosynthetic rate in green leaves of infected plants is not a suitable physiological selection criterion in breeding potatoes for tolerance to late blight.

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**Samenvatting**

*De invloed van Phytophthora infestans op de fotosynthese bij aardappel*

Bepalingen van netto fotosynthesesnelheden, bij verschillende aardappelrassen, werden uitgevoerd in het veld en onder geconditioneerde omstandigheden. De metingen werden gedaan aan groen blad van planten die in verschillende mate waren aangetast door *Phytophthora infestans*.

Infectie had geen significante invloed op de netto fotosynthesesnelheid bij lichtverzadiging, de efficiëntie van lichtbenutting bij lage lichtintensiteit, of de donkerademhaling. Het effect van *P. infestans* op de knolopbrengst van aardappelrassen lijkt uitsluitend veroorzaakt te zijn door een vermindering van groen bladoppervlak. Daarom is selectie van aardappelgenotypen met superieure handhaving van fotosynthetische activiteit bij aantasting, geen kansrijk veredelingsdoel.

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