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Originalarbeit

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Latent infection rate of potato seed tubers with *Phytophthora infestans* (Mont.) de Bary – an underestimated problem

Latente Infektionsrate von Pflanzkartoffeln mit *Phytophthora infestans* (Mont.) de Bary – ein unterschätztes Problem

Abstract

From 2007 to 2009 applying molecular methods a total of 17 batches of seed potatoes were tested for the rates of latent infections with *Phytophthora infestans* (Mont.) de Bary, the pathogen of late blight. The samples were chosen randomly from different certified European seed potato productions. The average infection rate of the seed potatoes tested was 11%. The highest occurring rate was 38%. The given data show that there is no significant correlation between late blight susceptibility-levels of varieties and rates of latent infestation. There were no statistically significant differences found between seed potatoes from organic and conventional production.

Key words: Late blight, tuber blight, stem blight, PCR-test, *Solanum tuberosum*

Zusammenfassung

In den Jahren 2007 bis 2009 wurde bei 17 zufällig ausgewählten Partien von zertifiziertem Kartoffel-Pflanzgut europäischer Herkunft die latente Infektionsrate mit *Phytophthora infestans* (Mont.) de Bary, dem Erreger der Kraut- und Braunfäule, anhand von molekularbiologischen Methoden bestimmt. Im Mittel waren 11% der Pflanzknollen befallen. Die höchste Infektionsrate einer Partie lag bei 38%. Ein signifikanter Zusammenhang zwischen der Braunfäuleanfälligkeit der Sorten und der erhobenen latenten Infektionsraten konnte nicht festgestellt werden. Auch gab es keine statistisch absicherbaren Unterschiede zwischen konventionell und nach den Richtlinien des ökologischen Landbaus erzeugten Pflanzgutpartien.

Stichwörter: Krautfäule, Braunfäule, PCR-Test, *Solanum tuberosum*

Introduction

Potato late blight is caused by the oomycete *P. infestans* and resembles one of the most important diseases in worldwide potato production. Especially early stem blight leads to high late blight infection pressure that can result in severe losses of quality and yield under favourable climatic conditions of cold and humid weather (Shtienberg et al., 1990). In most cases the source of an epidemic is the mycelium overwintering within tubers (Kadish and Cohen, 1992). When overwintering takes place in tubers on cull piles or in volunteers, the pathogen can grow and sporulate on these plants during the next growing season. The sporangia of *P. infestans* can be spread by wind and cause infections on other plants. The resulting infections on potato plant tops are so-called secondary infections, mainly on the leaves. More important nowadays is the overwintering in infected tubers in stor-

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age (Zellner, 2004). Optimal modern storage conditions prevent the pathogen from growing so no visible symptoms appear on the tuber. Such healthy looking, yet latently infected tubers are used as seed tubers, bringing the pathogen directly into the field and being the main cause for stem blight (Fig. 1). With high soil humidity the fungus can expand into the stem or, as Adler (2000) and Bässler et al. (2002 und 2004) showed in greenhouse experiments sporangia can be produced on the infected tubers, spread with soil water and infect stems and tubers of neighbouring plants, leading to primary stem blight in both cases. During the official certification process and the rejection of visibly infected tubers, latent late blight infestation is not assessed. As a result the seed tubers may look healthy, but they can still carry *P. infestans* right into the new planting sites, leading to an early primary outbreak of this disease. Thus the main goal of this three-year study was to analyse certified seed potatoes from all over Europe to identify the present rate of latent infestation and to make an estimation of the real threat caused by this phenomenon.

Methods

Seed tuber sampling
During the years 2007 to 2009 17 batches of European certified seed tubers were tested for latent infection with *P. infestans*. From each charge 47 tubers (respectively 94 in 2007) were randomly chosen and tested. Five batches were organically produced while the others originated from conventional production. No batch with high susceptibility for late blight on tubers could be tested, but 9 with medium (4-6) and 6 with lower (1-3) sensitivity towards *P. infestans* according to the Federal Plant Variety Office. For two samples no classification was available.

Processing of samples – Extraction of DNA
The surface of the tubers was thoroughly cleaned with a scrubber under running water. Each tuber was grinded in a juicer (HR 1861; Royal Philips Electronics, Eindhoven, Netherlands). 70-100 mg of the resulting pulp was put into a reaction tube and mixed with 100 µl Tris-buffer (10 mM, pH 8.0, Carl Roth GmbH, Karlsruhe, Germany). The samples were stored in the refrigerator at 7°C until further processing. Extraction of DNA was performed with the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). The DNA solutions were stored at -20°C until amplification by polymerase chain reaction (PCR).

Fig. 1. Latently infested seed tubers with *P. infestans* are the main reason for early symptoms of stem blight.

Detection of Phytophthora infestans via PCR
The extracted DNA of the pathogen was amplified with a primer setting recommended by Judelson and Tooley (2000) with the primers 5'-GAAAGGCATAGAAGGTAGA-3' (forward primer 08-3) and 5'-TAACCGACCAAGTAGTAAA-3' (reverse primer 08-4) giving a 258 bp amplicon. Concentration of the reaction-mixture was as following: 10 ng DNA/µl, 10% PCR-Buffer y (Qiagen GmbH, Hilden, Germany), 0.4 µM of each primer (Carl Roth GmbH, Karlsruhe, Germany), 2 mM $MgCl_2$ (Qiagen GmbH, Hilden, Germany), 200 µM deoxynucleotide triphosphates (Carl Roth GmbH, Karlsruhe, Germany) and 0,67 units TaqDNA polymerase (Qiagen GmbH, Hilden, Germany). Amplification was performed in a MJ Research PTC-200 thermal cycler using 96 µl well-plates. The reactions were incubated at 94°C for 30 sec followed by 35 cycles of 94°C (30 sec), 30 sec of 54°C for annealing and 60 sec at 72°C for elongation. Final elongation was performed at 72°C for 240 sec, before the reaction was held at 10°C.

5 µl of the amplified products were resolved by electrophoresis in 0.9% agarosis gels (Agarose NeeO, Carl Roth GmbH; Karlsruhe, Germany) in Tris-Borate-EDTA buffer (Sigma-Aldrich Chemie GmbH, Munich, Germany) and stained with 0.005% ethidium bromide (Carl Roth GmbH, Karlsruhe, Germany). 2.5 µl of a DNA-Ladder (Low range mass ruler, MBI Fermentas GmbH, St.Leon-Roth, Germany) was used as a standard. Images were captured digitally.

Results

The single results of the molecular detection of *P. infestans* in different batches of seed tubers are listed in Tab. 1.

In 2007 2 out of 5 tested batches showed latent infections on more than 10% of the seed tubers and one charge was without infection. The mean infection rate was 11.2%, meaning that on average every ninth tubers was infected.

The average rate of latent late blight was 12.7% in 2008 with no charge found free of infections and 4 out of 6 with infestation above 10%.

In 2009 2 out of 6 tested batches were free of latent infections and the same amount showed infestations rates above 10%. The mean infection rate was 9.2%.

The results from comparing different groups of batches are listed in Tab. 2, showing that the means of organically

Tab. 1. Percentage of latent infected potato seed tubers. (Susceptibility to *P. infestans* according to the Federal Plant Variety Office (1 = lowest, 9 = highest); SD: standard deviation)

Year	Variety	Organic	Susceptibility of tubers	Susceptibility of leaves	Latently infected [%]
2007	Agria I	Yes	5	4	37
	Agria II	No	5	4	11
	Bonza	No	Not classified	Not classified	6
	Cindy	No	3	5	0
	Melina	Yes	3	5	2
	Mean (SD)				11.2 (± 15.0)
2008	Agria	No	5	4	17
	Baril	Yes	Not classified	Not classified	17
	Ditta	No	4	5	2
	Krone	Yes	3	5	11
	Laura I	No	3	5	6
	Laura II	No	3	5	23
	Mean (SD)				12.7 (± 7.8)
2009	Agria	No	5	4	11
	Ditta I	No	4	5	0
	Ditta II	No	4	5	0
	Ditta III	Yes	4	5	2
	Laura	No	3	5	4
	Maxilla	No	5	5	38
	Mean (SD)				9.2 (± 14.7)

Tab. 2. Overall summary of the infestation rates of the 17 tested batches 2007-2009. (Susceptibility to *P. infestans* according to the Federal Plant Variety Office (1 = lowest, 9 = highest); SD: standard deviation)

	Number of tested batches	Mean infestation rate (SD)	t-test $p < 0.05$
Organically produced seed tubers	5	13.8 (± 14.4)	a
Conventionally produced seed tubers	12	9.8 (± 11.4)	a
Seed tubers with medium susceptibility on tubers (4-6)	9	13.1 (± 15.0)	a
Seed tubers with lower susceptibility on tubers (1-3)	6	7.7 (± 8.4)	a
Summary of all tested batches	17	11.0 (± 12.1)	

and conventionally produced seed tubers showed no significant differences (t-test, $p < 0.05$) in the average percentage of latent infected seed tubers.

There was no significant difference (t-test; $p < 0.05$) between seed tubers from medium (4-6) and lower (1-3) susceptibility groups to tuber blight.

A comparison of seed tubers regarding the susceptibility to late blight on leaves was not futile since all tested tubers were medium susceptible (4-6).

The overall average of infestation throughout all 17 tested seed tuber batches was 11%. The highest rate of latent infestation was 38%. 17.6% (3 out of 17) were tested negative on *P. infestans*.

Discussion

The given data show that using certified, symptom less seed tubers is no guarantee for having really healthy tubers, but bears the significant risk of bringing *Phytophthora infestans* to the fields with the new seed tubers. The chance of having latent infections seems not related to whether the potatoes are obtained from organic or conventional seed tuber production. The susceptibility of the variety does not give a hint on the probability for an infestation. Latent infections with *Phytophthora infestans* in potato seed tubers are considered to be a general problem. Because of the high risk of infected seed tubers con-

trol measures are highly recommended. In conventional potato farming infections can be controlled by means of systemic fungicides that spread into the plant tissue and can reduce the growth of the fungus within the plant. In organic farming the treatment of seed tubers with copper is a possibility to reduce primary infections. However, the use of protective fungicides in conventional production proves to be more efficient. It is important to keep the infection rate as low as possible as they initiate new infections in the field during the following year. In our further research we will therefore focus on establishing scientifically based methods to reduce the latent infection rate of *P. infestans* in potato seed tubers.

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