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Double Setting of Potato Seed Tubers as a New Approach to Research Primary Stem Blight (*Phytophthora infestans* (Mont.) de Bary)

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Abstract The double setting of two seed tubers is introduced as a new method for research on primary stem blight of potato, *Solanum tuberosum L.* caused by *Phytophthora infestans* (Mont.) de Bary). The principal focus of this experimental layout is the planting of a healthy seed tuber adjacent to an inoculated one, termed double setting. The infected tuber serves as a source of inoculum, enabling the disease to spread to the neighboring plant, where stem infections occur. The new method was compared to the common procedure of direct inoculation of tubers. The rate of emergence using double setting was significantly higher compared to direct inoculation. Additionally, stem blight was more intense and generally occurred earlier. Direct inoculation proved to be less suitable for research where the occurrence of higher percentages of stem blight symptoms is necessary. Further more, the double setting method generally permits insight into the spread of the disease from latent infected tubers to healthy ones under the natural conditions within the potato hill.

Resumen Se presenta el duplicado de dos tubérculos semilla como nuevo método de investigación en tizón de tallo primario de papa, *Solanum tuberosum L.*, causado por *Phytophthora infestans* (Mont.) de Bary. El enfoque principal de este planteamiento experimental es la siembra de un tubérculo semilla sano junto a otro inoculado, que le llamamos duplicado. El tubérculo infectado sirve como fuente de inoculo, permitiendo que la enfermedad se disperse a la planta vecina, donde se presenta la infección del tallo. El nuevo método se comparó con el procedimiento común de inoculación directa de los tubérculos. El nivel de emergencia del duplicado fue significativamente mas alto comparado con la inoculación directa. Además, el tizón del tallo fue mas intenso y generalmente se presentó más temprano. Se evidenció que la inoculación directa fue menos deseable para investigación cuando se necesita la incidencia de porcentajes mayores de síntomas de tizón del tallo. Aún más, el método del duplicado generalmente permite discernir sobre la dispersión de la enfermedad de tubérculos con infección latente a los sanos bajo las condiciones naturales al interior del surco.

Keywords Late blight · Primary infection · Artificial inoculation · *Solanum tuberosum*

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Introduction

Late blight, caused by the oomycete *P. infestans* is one of the most important diseases in worldwide potato production. Under cool and humid climatic conditions this pathogen can cause severe losses in crop yield (Shtienberg et al. 1990). In most cases overwintering mycelia within tubers is the origin of the epidemic (Kadish and Cohen 1992). Two distinct modes of infection can be observed. If the overwintering takes place in volunteers or in tubers on cull piles, the pathogen can infect and grow on these plants in the next growing season. From these plants sporangia of *P. infestans* are spread by the wind. Infections caused by

Fig. 1 Potato leaves of the variety 'Agria' with secondary infections of *P. infestans*

Fig. 2 Potato plant (variety 'Agria') showing intense stem blight caused by *P. infestans*

these air borne sporangia on potato tops are so-called secondary infections (Fig. 1). The appearance of these kinds of infection can be predicted by forecasting models and managed by timely application of fungicides. Overwintering can also take place in infected tubers in storage. During the optimal modern storage conditions the tubers, as well as the pathogen within, remain physiologically inactive. Thus no visible symptoms are developed and the latent infected tubers are used as seed tubers. These latent infected seed tubers are a major source for primary infections (Zellner 2004), since the pathogen is brought directly into the field. According to greenhouse experiments of Adler (2000) and Bäßler et al. (2002 und 2004) sporangia can be produced on the infected tubers at high humidity. The sporangia are spread with soil water and release zoospores, which actively swim, to infect stems and tubers of neighboring plants. As a result primary stem blight occurs (Fig. 2). Unlike secondary infections the appearance of primary stem blight cannot be calculated. Especially in organic potato farming these infections are a serious threat, since no curative fungicides are available to remove the pathogen from the host tissue. Thus an early establishment of the disease in the standing crop has to be avoided at all costs. Therefore further research on primary stem infections and the processes taking place in the potato hill is utterly necessary to investigate. Under natural conditions, stem blight only appears on a small percentage of plants in the field. Nevertheless, these few infection sites are the origin of the epidemic within the standing crop. A prerequisite for successful study of primary infection as performed by Keil et al. (2009) is a consistent and uniform rate of infection of the seed tubers. A low rate of stem blight reduces the informative value of an experiment. Since the natural infection is non-uniform and suppressed by plant defense mechanisms, artificial inoculation of the tubers is necessary. Depending on susceptibility, climatic conditions and aggressiveness of pathogen isolate, the induced outbreak of the disease can cause the inoculated tuber to die off before emergence, leading to non-uniform crop stand development. If too many plants are lost the results will not be reliable due to shortage of data points. The main goal of this study was to get a high initial rate of infestation combined with a high emergence of plants to evaluate.

Methods

Plant Material

Potato varieties 'Agria' and 'Quarta' were used from 2005–2007 and 'Agria' and 'Laura' in 2008 and 2009. These combinations of potato varieties were selected so the test plants could be differentiated phenotypically. While 'Agria' showed an intense purple coloration of the stem base, the

Table 1 Inoculation scheme for the field trials 2005–2009 with *Phytophthora infestans*

Inoculation technique	Inoculation of seed tubers	
	'Agria'	'Quarta' / 'Laura'
Non-treated control	no inoculation	no second tuber
Direct inoculation of 'Agria'	50 zoospores	no second tuber
Double setting (indirect inoculation of 'Agria')	no inoculation	200 zoospores

Fig. 3 Inoculation of seed tuber (variety 'Agria') by injection of *P. infestans* zoospores with a pistol grip syringe (Socorex Isba S.A., Switzerland)

stem of 'Quarta' was completely green. 'Laura' can be identified by its red leaf veins. Only certified seed tubers were used.

Pathogen and Inoculation

P. infestans (isolates 31 and 57, Bavarian State Research Centre for Agriculture, Freising, mating type A1).was plated on V8-Agar at 17°C. Before inoculation, the pathogens were propagated on tuber slices to maintain aggressiveness. Sporangia were rinsed off with sterile water and filtrated through a gauze cloth to remove mycelium. To induce the release of zoospores the suspension was kept at 10°C for 4 h. Subsequently the concentration of zoospores per milliliter was adjusted as desired. To prevent the decay of the single infected tubers 'Agria' were inoculated with 50 zoospores only to get weak infections, while for the double settings severe infections of the corresponding 'Quarta' or 'Laura' had to be ensured by inoculation with 200 zoospores to promote the spread of the disease from the infected tuber to the neighboring one. (Table 1). For inoculation, 50 µl of the corresponding suspension, containing the desired number of zoospores, was injected into the tubers by the use of a pistol grip syringe (Socorex Isba S.A., Switzerland) as shown in Fig. 3. The syringe (length 32 mm; outer diameter 0.7 mm) was inserted fully into the tissue at a random location of the tuber and slowly retracted. Then the zoospore-solution was injected into the resulting cavity. Inoculation was generally performed one to three days before planting.

Plot Setup and Double Setting of Tubers

Eleven field trials were conducted between 2005 and 2009 at the Bavarian sites of Puch (para-brown earth, heavy soil) and Straßmoos (brown earth, light soil). Each trial had three to four replications, each one consisting of six rows of 20 (Puch) or 17 (Straßmoos) plants. Seed-spacing was 30 cm within and 75 cm between the rows. The tubers were set by hand and the dam was formed afterwards, so that the planting depth within the dam was about 15 cm for all tubers.

For the double setting, two touching seed tubers of different varieties were planted into the same planting hole. Adjacent to a healthy 'Agria' an artificially inoculated seed tuber (either 'Quarta' or 'Laura') was set. In the single setting experiment, only single tubers of the variety 'Agria' were planted. For the conventional method of direct inoculation they were injected with 50 zoospores, while in

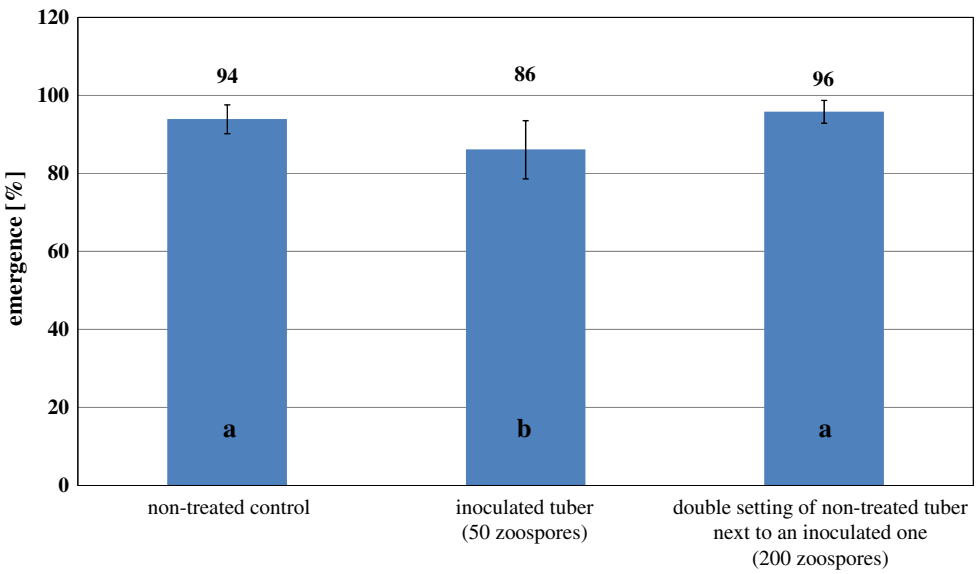

Fig. 4 Rate of emergence [%] of potato plants (variety 'Agria') throughout 13 field trials (2005–2009); For non-treated control and direct inoculation single tubers were planted, while for double setting a non-treated tuber was planted adjacent to an inoculated one in the same planting hole. Number of zoospores of *P. infestans* used for inoculation is given in brackets. Treatments with different lowercase letters are significantly different (p=0.01)

Table 2 Average proportion [%] of all infections from 11 field trials (2005–2009) throughout 2 weeks after initial outbreak of stem blight

Inoculation technique	Initial outbreak		1 week after initial outbreak		2 weeks after initial outbreak	
Control (single non-treated seed tuber)	12.0	a	25.1	a	28.7	a
Direct inoculation of single seed tuber	12.2	a	13.0	a	13.0	a
Indirect inoculation by double setting of seed tubers	75.8	b	61.9	b	58.3	b
LSD (p=0.05)	29.6		30.7		26.1	

Treatments labeled with different lower-case letters are significantly different (p<0.05)

the control plots the tubers remained non-treated as control (Table 1). After emergence (four to five weeks after planting), the frequency of stem blight was evaluated at weekly intervals for 20 plants per replication, by checking the first 10 plants of the third and fourth row for the number of plants showing symptoms on their stems. For comparability, only the 'Agria' was considered. Statistical analysis was performed by comparing the mean frequency of the single years by Fisher´s LSD. Since there was no interaction between two experimental sites and 5 years of testing, the results for 11 trials were combined.

Results

With the standard method of direct tuber inoculations, the rate of emergence was significantly (P<0.05) lower compared to the non-treated control, and the double setting treatment (Fig. 4). Emergence was not influenced by the presence of inoculated tubers adjacent to healthy ones, and emergence in double setting treatment was not different (P>0.05) from the control.

Table 2 shows to which degree the three inoculation techniques contributed to primary infections within the first 2 weeks after the initial appearance of stem blight. By double setting, 75.8% of all initial infections were achieved. Direct inoculation caused the least primary stem blight. In most cases the visual symptoms occurred earlier when the new technique of double setting was employed. One week after initial outbreak, 61.9% of the infected plants were in the double setting treatment, which was higher (P<0.05) than in direct inoculation and control treatments. Similarly, 2 weeks after initial outbreak, the stem blight incidence (58.3%) was higher (P<0.05) with double setting treatment than control or direct inoculation treatments. Throughout the three evaluation periods, the highest stem blight incidences were always recorded for double setting, and the lowest for direct inoculation treatment.

The average frequency of plants showing stem blight is shown in Table 3. At the initial outbreak the percentage of visually infected plants was significantly higher using double setting compared to the control or the direct inoculation technique. One week later an average of 7.9% of the double set plants showed stem blight which was higher though not statistically different compared to 2.3% if the tubers were inoculated directly. At the third evaluation, 2 weeks after the first occurrence of the infection, no significant differences were found between the three inoculation techniques, but still the lowest percentage of visually infected plants was found at direct inoculation. At this point most of new infections are not soil-borne, but are mostly from sporangia produced on established infection sites within the standing crop. Thus these infections are considered as secondary infections, even as they affect the stem, and have no significance for the research of primary stem blight.

Discussion

For consistent and reliable research on primary stem blight the occurrence of symptoms has to be ensured. In order to be able to compare the effectiveness of certain fungicide measurements or seed treatments (Keil et al. 2009, Benker and Zellner 2008) it is crucial to get as many primary stem infections as possible. If less than 3% of the plants show

Table 3 Average percentage of plants showing stem infections [%] within 2 weeks after initial outbreak of stem blight in 11 field trials (2005–2009)

Inoculation technique	Initial outbreak		1 week after initial outbreak		2 weeks after initial outbreak	
Control (single non-treated seed tuber)	0.3	a	2.9	a	12.2	a
Direct inoculation of single seed tuber	0.3	a	2.3	a	9.9	a
Indirect inoculation by double setting of seed tubers	1.8	b	7.9	a	15.5	a
LSD (p=0.05)	0.8		6.3		15.1	

Treatments labeled with different lower-case letters are significantly different (p=0.05)

stem infections within 1 week, as it is for direct inoculation, the results of the experiments have hardly any informative value and are not reliable. As soon as 2 weeks after the initial outbreak, new infections can not be considered as primary stem blight anymore, and may result from airborne sporangia. Thus, an adequate number of infected stems have to develop within 1 week after the outbreak to get any reliable results. This can not be accomplished by the use of naturally infected tubers, since only a very small percentage of the emerging plants show symptoms (Hirst 1955). Artificially infected seed pieces must be used. But even if directly inoculated tubers are used, as it is shown in our field trials, less than 2.5% of the plants show infected stems for evaluation within 1 week after the initial outbreak. Besides, the climatic conditions might not be suitable for stem blight to develop (Bäßler et al. 2004), and also if the density of inoculum is too high the majority of the tubers may rot quickly (Powelson et al. 2002), and fewer plants left for evaluation. This leads to a low frequency of stem blight in the plot, often outnumbered by the non-treated control. However, if the inoculum density is too low no infection occurs. Since the infection severity is difficult to manage, and the outcome is often unpredictable, generally direct inoculation is not reliable. This can be significantly improved by the double setting method described here. The healthy tubers showed good rate of emergence though with some frequency of stem blight. These healthy plants were presumably infected by being next to the heavily inoculated neighboring tubers. The release of sporangia and zoospores and their transmission to healthy plants will depend on suitable environmental conditions (Sato 1980). Thus, a natural infection process is simulated leading to a high frequency of infected plants. The double setting method offers a new way to set up experiments and research the spread of pathogen from latent infected tubers to neighboring plants. It has proven to be effective and thus has been successfully used for several years at the Bavarian State Institute for research on primary stem infections (Benker and Zellner 2008, Keil et al. 2009)

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