Twelfth EuroBlight workshop
Arras (France), 3-6 May 2010

Effect of spore density, cultivar resistance and *Phytophthora infestans* isolate on tuber blight under field conditions.

A. EVENHUIS, P.J. VAN BEKKUM, AND G.J.T. KESSEL

Plant Research International, Wageningen University and Research Centre.
PO Box 69, 6700 AB Wageningen, The Netherlands

SUMMARY
Survival of *P. infestans* in soil was limited to 5 weeks at high inoculum density. With a ten fold dilution of the infection pressure survival of *P. infestans* in soil was limited to two weeks. Differences between isolates concerning survival in the soil were small. From these experiments we conclude that survival of *P. infestans* in soil depended on spore density rather than *P. infestans* isolate used.
Tuber blight infection rate depends on density of the sporangia in the soil, tuber blight resistance of the cultivar and to a lesser extent the *P. infestans* isolate used. Isolate of type EU 13 A2 was not more aggressive to tubers than IPO 428-2, regardless of the resistance level of the cultivar used. A mixture of isolates was found to be more aggressive than two single isolates tested on cultivar Bintje only. Thus measures to avoid infection of the soil with *P. infestans* sporangia lower the tuber blight infection risk, especially on the susceptible cultivar Bintje.

KEYWORDS
Solanum tuberosum; potato; late blight;

INTRODUCTION

Sporangia of *P. infestans* can be washed through the soil to the tubers (De Bary, 1863). Subsequently tubers can be readily infected with *P. infestans*. The number of spores washed to the soil depends on the late blight epidemic developing in the canopy and weather conditions, especially rainfall. Tuber blight incidence further depends on tuber blight resistance of the cultivar used. At present the *P. infestans* population in The Netherlands is dominated by isolates of the EU 13 A2 type. Isolates of type EU 13 A2 are considered more aggressive to potato foliage (Lees *et al.*, 2009) than isolates of the old population, but this does not necessary apply to tubers also. The aim of the experiments was to assess the effect of *P. infestans* isolates, cultivar resistance and spore density on tuber blight incidence, without the confounding effect of a late blight epidemic in the foliage.

PPO-Special Report no. 14 (2010), 79 - 84

MATERIALS AND METHODS

Treatments
Potato cultivars Bintje, Agria and Seresta were planted in spring 2009 on a heavy clay soil at Wageningen. Tuber blight resistance ratings according to the National list are 4.5, 7.5 and 8 respectively. To avoid late blight in the foliage the potato crop was sprayed regularly with a fungicide containing active ingredients cymoxanil + mancozeb. Desiccation of the crop took place at the end of August. Potato tubers were harvested by hand on September 21st.

Preparation spore suspension
Isolate IPO 428-2, an isolate of type EU 13 A2 and a mixture of 16 isolates were used to inoculate the experiments. The isolates were kept in liquid nitrogen storage until use. During the experiments the isolates were maintained alternating on detached leaves and slices of potato of cv. Bintje in a climate room at 15°C with a 16 hour photoperiod. Mycelium from slices were transferred to leaves on 1.5% water agar (WA) in a Petri dish with vents (Ø = 9 cm) in one week and parts of infected leaves were transferred under a slice in a Petri dish with vents (Ø = 9 cm) in the other week. Potato leaves were obtained from plants grown in a greenhouse at 18°C. To produce large amounts of sporangia wetted filter paper was placed in a large tray of 530x320x60 mm. A grid was placed on the wetted paper and wetted oases was placed at both sides of the tray. Detached leaves were put in the oases and inoculated with *P. infestans* isolates. The tray was covered with a transparent plastic bag and placed in the climate room. For each isolate treatment 16 trays with infected leaves were used.
Spore suspensions were made by washing off sporangia from infested leaves with cold tap water. Spore density of the suspension was determined with a coulter counter (Beckman Coulter, inc). The highest spore density (100%) was prepared, and subsequently a ten fold dilution was made (10%). The freshly made spore suspension (50 ml) was added directly to the top of the ridge, between the potato plants in the field. Each treatment consisted of 18 potato plants being inoculated, next to stem upon the top of the ridge.
The experiment was inoculated three times during the season on 15 July, 5 August and 9 September. Each time inoculation took place in plots which were not previously inoculated.

Survival of sporangia in the soil
Infectivity of the spores was measured in time. Soil samples were taken from the top of the ridge, where previously the soil was inoculated with *P. infestans*. A modified tuber-slice test as described by Lacey (1965) was used to estimate survival of sporangia. The trays were placed in a climate room at 15°C with an 16 hour photoperiod. After one day slices were cut in eight pieces (octants) and separated with a single edge razor blade and placed back in the climate room. Octants were examined six days later for infection by *P. infestans*. Octant infection rate was calculated by the dividing the number of infected octants with the total number of octants. If no infection of octants occurred for each replicate on two consecutive sample dates it was assumed that sporangia did not survive the treatment.

Tuber blight assessment
Tuber blight assessments were made shortly after harvest and after incubation for three weeks in a climate room at 15°C in the dark. Both number and weight of the potatoes were assessed. Infected tubers were removed at the first assessment. These tubers were counted and weighed. At the end of the incubation period tubers were washed and a second tuber blight assessment was made. The number and weight data from both assessments were combined and the percentage tuber blight was calculated.

Statistical analyses

Three experiments were carried out. Each experiment was laid out as split plot design, with inoculation date and cultivar randomized as a complete block design. Within these plots two treatment factors, being the isolates and the inoculum density to be tested were randomized. Each experiment was carried out with three replicates. Each experiment was inoculated one time during the season. Inoculation dates were 15 July, 5 August and 9 September. Since differences between inoculation date (experiments) on tuber blight incidence and octant infection rate were marginal compared to the other treatment effects, data over the three experiments were pooled.

Analysis of Variance (ANOVA) was performed on tuber blight incidence based on weight, measured per experimental plot, using Genstat release 12.1 (Payne *et al.,* 2002).

RESULTS

Octant infection rates tended to approach zero in approximately 15 days at a low inoculum density (10%). At a high inoculum density (100%) octant infection was found up to five weeks after inoculation (Figure 1), depending on the inoculum source. Differences between isolates concerning survival in the soil, measured as octant infection rate, were small compared to density of sporangia applied.

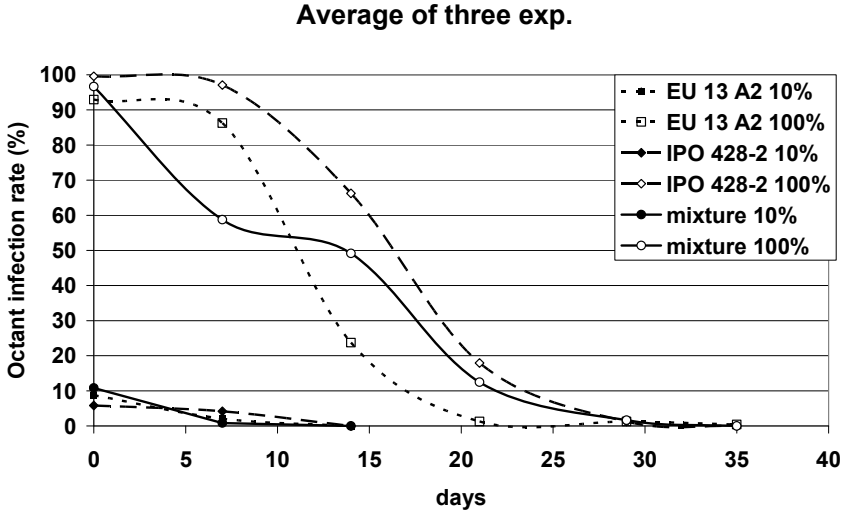

Figure 1. *Octant infection rate depending on P. infestans isolate, spore density applied and time after inoculation (days).*

No foliar or stem blight occurred in the field during the experiment.

Average tuber blight incidence increased significantly from 0.5 to 1.5 (P<.001) when the inoculum density was increased from 10% to 100%. A significant (P <.001) cultivar and inoculum density interaction was observed (Table 1). On average the tuber blight incidence after inoculation with a mixture of isolates was 1.5% and was significantly (P=0.05) higher than after inoculation with the single isolates EU 13 A2 and IPO 428-2. Pair wise comparisons between treatments are given in Table 2.

Table 1. *Average tuber blight incidence after inoculation of the ridge with P. infestans isolates at two spore densities.*

	Cultivars					
Spore density	Agria		Bintje		Seresta	
10	0.9	. b . [1]	0.4	a b .	0.1	a . .
100	1.4	. b .	2.5	. . c	0.6	a b .

[1]: *Treatments followed by different characters differ significantly (P=0.05) from each other, based on angular transformation. Represented data are not transformed.*

Table 2. *Percentage tuber blight after inoculation of the ridge with P. infestans isolates at two spore densities.*

	Spore	Cultivars					
Isolate	density	Agria		Bintje		Seresta	
EU 13 A2	10	0.7	a b c d . . .[1]	0.3	a b c	0.1	a . . . . .
EU 13 A2	100	0.8	a b c d . . .	1.8	. . . . . f .	0.8	. b c d e f .
IPO428-2	10	0.7	a b c d e . .	0.0	a	0.2	a b . . . . .
IPO428-2	100	1.6	. . c d e f .	1.7	. . . . e f .	0.3	a b . . . . .
Mixture	10	1.4	. . . d e f .	1.1	. b c d e f .	0.2	a b . . . . .
Mixture	100	1.7	. . . d e f .	4.0	. . . . . g	0.7	a b c d e . .

[1]: *Treatments followed by different characters differ significantly (P=0.05) from each other, based on angular transformation. Represented data are not transformed.*

DISCUSSION

Octant infection rate is a means to estimate survival of *P. infestans* in soil. Survival of *P. infestans* in soil was limited to 5 weeks maximum at high inoculum density in our experiments, which was in the range described by others (Murphy, 1922; Zan, 1962; Lacey 1965, Andrivon, 1994). Survival of *P. infestans* spores was 64 days in a previous experiment in a clay soil at Wageningen (Evenhuis *et al.*, 2006). In our experiments in 2009 inoculum was poured on to the soil whereas in 2004 the inoculum was placed in the soil in mesh bags. The survival in 2009 was possibly under estimated due to the inoculation method and subsequent sampling technique used.

Octant infection rate at the start of the experiment depended on inoculum density applied. Subsequently the duration of survival of *P. infestans* in the soil was influenced by the applied *P. infestans* inoculum density as well. The effect of *P. infestans* isolate used on spore survival was small. No differences in survival rate in soil of isolates of the US clonal lineages US-8 and US-11 were found (Porter & Johnson, 2007).

Tuber infection depends on several factors leading to the presence of *P. infestans* sporangia in the soil (Lapwood, 1977). On average tuber blight incidence was significantly higher after inoculation with a higher inoculum density, suggesting a quantitative relation between the presence of sporangia in the soil and tuber blight. Over all tuber blight incidence was low, possibly because a single inoculation has been carried out in each plot. Sporangia can be washed to the soil on several days, providing rain fall occurs during sporulation events, in agricultural practice, leading to a higher inoculum pressure.

P. infestans was inoculated on the ridge directly in our experiment. No late blight developed in the potato foliage. Thus the inoculum pressure applied was due to the inoculation of the potato ridge only. Thus treatments (isolate used and spore density applied) did not influence each other. Also the confounding effect of the resistance level of the cultivar to foliar blight was taken out of the equation. Furthermore the weather circumstances necessary for late blight development in

the foliage and wash down of spores from the foliage to the ridge was avoided. Water containing sporangia of *P. infestans* washed into the soil immediately, mainly through cracks in the clay soil. Further transport of the sporangia towards the tubers was not facilitated but depended upon the natural rain fall during the experiments. Thus the effect of inoculum density, isolate and cultivar resistance on tuber blight could be studied in a field situation.

Actual tuber blight infection, apart from the presence of sporangia, is dependent on soil moisture content and the resistance of the cultivar (Lapwood, 1977). Differences between isolates were less conspicuous. The isolate of type EU 13 A2, predominant in the Dutch *P. infestans* population (data not published), was not more aggressive in causing tuber blight than isolate IPO 428-2. IPO 428-2 was collected in 1992 and represents the old population, although this isolate is known to be aggressive towards tubers (Flier *et al.,* 2001). Isolate IPO 428-2 and the isolate of type EU 13 A2 were also included in this mixture of 16 isolates. The mixture of 16 isolates was more aggressive to tubers than both single isolates, when tested on cultivar Bintje. The total inoculum density applied in the mixture was the same as with the single isolates. Therefore one or more of the isolates in the mixture are responsible for the increased tuber blight incidence.

Differential interactions in tuber blight attack between potato cultivars and *P. infestans* isolates was found previously (Flier *et al.,* 2001). In our experiments no clear significant (P=0.062) differential interaction was found.

CONCLUSIONS

From these experiments we conclude that survival of *P. infestans* in soil depended on spore density rather than *P. infestans* isolates used.

Tuber blight infection rate depends on density of the sporangia in the soil, tuber blight resistance of the cultivar and to a lesser extent the *P. infestans* isolate used. The isolate of type EU 13 A2 was not more aggressive to tubers than isolate IPO 428-2 originating from 1992, regardless of the resistance level of the cultivar used. A mixture of isolates was found to be more aggressive than two single isolates tested on cultivar Bintje only. Measures to avoid infection of the soil with *P. infestans* sporangia lower the tuber blight infection risk, especially when the susceptible cultivar Bintje is grown.

ACKNOWLEDGEMENTS

The research was funded by the Dutch Ministry of Agriculture, Nature and Food Quality and was part of the Netherlands initiative on potato late blight.

REFERENCES

Andrivon, D., 1994. Dynamics of the survival and infectivity tot potato tubers of sporangia of *Phytophthora infestans* in three different soils. Soil Biol. Biochem. 26: 945-952

De Bary, A., 1863. Recherches sur le devolpment de quelques champignons parasites. Ann. Sci. nat. Bot. 20: 5-148.

Evenhuis, A., Kessel, G.J.T., Van Bekkum, P.J., 2006. Epidemiology of *P. infestans* in relation to tuber bligth. Survival of *P. infestans* sporangia in fields soils. PPO special report 11: 223-227.

Flier, W.G., Turkensteen, L.J., Van Den Bosch, G.B.M., Vereijken, P.F.G., Mulder, A. 2001. Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different levels of blight resistance Plant Pathology 50 (3): 292-301.

Lacey, J., 1965. The infectivity of soils containing *Phytophthora infestans*. Annals Applied Biology 56:363-380.

Lapwood D.H., 1977. Factors affecting the field infection of potato tubers of different cultivars by blight (*Phytophthora infestans*). Annals of Applied Biology 85: 23–45.

Lees, A.K., Cooke, D.E.L., Stewart, J.A., Sullivan, L., Williams, N.A., Carnegie, S.F., 2009. *Phytophthora infestans* population changes: implications. PPO Special Report 13: 55-60.

Murphy, P.A. 1922. The bionomics of the conidia of *Phytophthora infestans*. Sci. Proc. R. Dublin. Soc. 16: 442-466

Porter, L. D., Johnson, D. A. 2007. Survival of sporangia of new clonal lineages of *Phytophthora infestans* in soil under semiarid conditions. Plant Disease 91: 835-841.

Zan, K. 1962. Activity of *Phytophthora infestans* in soil in relation to tuber infection. Trans. Br. Mycol. Soc. 45: 205-221