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The role of the seed tuber in determining partial resistance to potato blackleg caused by *Erwinia* spp.

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Abstract

In 1991 and 1992, 12 potato cultivars were screened at two locations for resistance to blackleg, after vacuum infiltration of the seed with *Erwinia carotovora* subsp. *atroseptica* or *E. chrysanthemi*. Cultivar differences for resistance to *E.c.* subsp. *atroseptica* and *E. chrysanthemi* were found which were consistent over locations and years. Seed tubers of the same cultivars were also screened for resistance to both *Erwinia* spp. by using a tuber slice inoculation method. Correlation coefficients for comparisons between resistance to blackleg in the field and tuber tissue resistance under aerobic or anaerobic conditions were not significant. This could partly be explained by drastic changes in relative tuber tissue resistance of the cultivars within a 5 weeks period after planting in the field. Presprouting of seed tubers in diffuse daylight had a less pronounced effect on relative tuber tissue resistance than planting in the field. Monitoring the process of mother tuber decay during the growing season of 1993 after vacuum infiltration with *E.c.* subsp. *atroseptica* and *E. chrysanthemi* revealed that cultivars differed in the extent to which these bacteria enhanced the process of mother tuber decay. These differences partly explained the cultivar differences for resistance to blackleg in the field.

Abbreviations: *Eca* = *Erwinia carotovora* subsp. *atroseptica*; *Ech* = *Erwinia chrysanthemi*; NOP = Noordoostpolder; Wag = Wageningen

Introduction

The use of seed potatoes that are latently infected with pectolytic *Erwinia* spp. can result in non-emergence of plants, stunting or wilting, and predominantly in blackleg of stems [Pérombelon and Kelman, 1987], especially under conditions that favour the multiplication of these bacteria [Pérombelon, 1992]. Although the bacteria do not seem to reduce yield significantly in temperate regions, infected tubers can under sub-optimal conditions cause severe losses due to rot in storage or due to blackleg associated symptoms when exported to areas were growing conditions are more favourable for the bacteria. National seed inspection services consider the occurrence of blackleg during seed production as an indication of contamination of the seed. Even low incidence of blackleg leads to declassification of the seed produced [Young, 1990] and as a consequence to significant economical damage to the seed tuber producers. Breeding for resistance to blackleg may contribute both to a reduction of the problem for seed producers and ware potato growers. Genetic

variation for resistance has been identified both in cultivated *Solanum tuberosum* [Zadina and Dobiáš, 1976; Hidalgo and Echandi, 1982] and in species of the *Solanum* section *petota* [Soest, 1983; Lojkowska and Kelman, 1989].

Attempts to breed for blackleg resistance will involve the screening of large numbers of clones. Methods are only suitable if they are easy to perform, applicable to early vegetative generations and yield reproducible and quantitative data that show good agreement with the resistance of the clones in farmers' fields. Numerous methods for screening of plants in the field [Hossain and Logan, 1983; Lapwood and Legg, 1983; Lapwood and Gans, 1984; Lapwood and Read, 1986a; Gans *et al.*, 1991], or in the glasshouse [Munzert, 1975; Hidalgo and Echandi, 1982; Lapwood and Read, 1986b; Lojkowska and Kelman, 1989] have been described. Field tests correspond most to field conditions but they are expensive to perform and not feasible when only a small amount of seed is available. Since rotting of the mother tuber is an essential step during blackleg development [Pérombelon and Kelman, 1980], it was thought that selection for resistance to blackleg might be carried out by screening for tuber tissue resistance in the laboratory. Of the many methods published [see Allefs *et al.*, 1995], the tuber slice inoculation method for screening of tuber tissue resistance as originally described by Lapwood *et al.* [1984] is one of the most extensively studied screening methods. It has been shown that for *Erwinia carotovora* subsp. *atroseptica (Eca), E.c.* subsp. *carotovora* and to a lesser extent also for *E. chrysanthemi (Ech)*, this method generates reproducible data under different experimental conditions [Lapwood *et al.*, 1984; Lapwood and Read, 1985; Wastie *et al.*, 1988; Allefs *et al.*, 1995]. The tests are much easier to perform than field tests, yield quantitative data and to some extent seem suitable for screening first generation tubers [Allefs *et al.*, 1995], but as far as known from literature, the relation between the results obtained in the tuber slice test and blackleg resistance in the field has not been studied. We studied this relation by screening a range of 12 cultivars for blackleg resistance in the field on two locations in two years and by comparing the results with tuber tissue resistance of the same cultivars as determined with the tuber slice inoculation method. The latter was also used for studying the effect on tuber tissue resistance of presprouting of seed potatoes and their growth in the field. Finally, the process of mother tuber decay after inoculation with *Eca* and *Ech* was followed under field conditions and the results related to both blackleg and tuber tissue resistance.

Materials and methods

Cultivars Agria, Alcmaria, Amazone, Arinda, Bintje, Désirée, Hertha, Karnico, Kondor, Morene, Producent and Venouska were chosen for this study because, over a range of years, they differ markedly for the percentage of seed potato fields that is declassified upon blackleg incidence. Furthermore, they represent the early, main and late maturity classes. The seed tubers used in the experiments were 40–50 mm in diameter and harvested from the same field in each of the years 1990 to 1992 with two exceptions. For the field experiments of 1991, tubers of Venouska and Morene were from one different source. For the main field experiment of 1993, tubers of Karnico and Producent were each from a different source. After harvest, tubers were stored at 4 °C until testing.

Bacterial strains and preparation of inoculum

Eca IPO 161 and *Ech* IPO 502 were obtained from the DLO-Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands. *Eca* was grown on Bouillon Agar medium containing 8 g l^{-1} 'Lab-Lemco' Broth (Oxoid), 5 g l^{-1} NaCl and 15 g l^{-1} agar. *Ech* was grown on Growth Factor medium containing 0.4 g l^{-1} K_2HPO_4, 0.05 g l^{-1} $MgSO_4.7H_2O$, 0.1 g l^{-1} NaCl, 0,5 g l^{-1} NH_2PO_4, 1 g l^{-1} glucose, 3 g l^{-1} Yeast Extract (Oxoid) and 15 g l^{-1} agar. Bacteria were cultured for 48 h at 27 °C, subsequently suspended in sterile water and centrifuged for 5 min at 4500 rpm. The bacterial pellet was resuspended and the concentration determined using a standard curve for each of the species relating colony forming units (CFU) to optical density at 500 nm. Bacteria were diluted to 1×10^9 CFU ml^{-1} in sterile water for tuber slice inoculation and to 3×10^7 CFU ml^{-1} (*Eca*, and *Ech* in 1991) or 2×10^7 (*Ech* in 1992

and 1993) in tap water for vacuum infiltration of tubers. Concentrations of inoculum for vacuum infiltrations were checked by dilution plating.

Resistance to blackleg

Seed tubers of the 12 cultivars were vacuum infiltrated with either tap water, *Eca*, or *Ech* and planted at two locations in The Netherlands. At Wageningen (Wag) experiments were carried out on a river type of heavy clay soil whereas in the Noordoostpolder (NOP) experiments were planted on a very light sandy soil. At both locations, a split plot design was used with four replicates which consisted of three randomized plots, one for each of the infiltration treatments. Plots consisted of 12 randomized subplots, one for each cultivar. Subplots consisted of two rows of eight plants.

Vacuum infiltration of the tubers was carried out by submergence into the inoculum at a pressure of 5 kPa for 10 min, followed by a period of recovering for 10 min at atmospheric pressure. In both years, one bacterial suspension of each of the bacteria was used eight times for inoculation of the tubers of one replicate which, per location, were treated in random order. Tubers were air dried overnight at room temperature and planted (Wag, 1991) or stored at 4 °C during one (NOP, 1991), two (Wag, 1992) or three weeks (NOP, 1992) until planting. Planting dates were May 3 and 13 at Wag and May 8 and 19 at NOP in 1991 and 1992 respectively. Trials at Wag were overhead irrigated with approximately 280 mm between the 12th of July and 3rd of August in 1991 and between the 18th of June and 5th of August in 1992. No irrigation was applied to the trials at NOP.

Non-emergence of tubers was scored once during the season and plants showing blackleg associated symptoms three and four times in 1991 and 1992, respectively. Analysis of variance (ANOVA) was carried out for the percentage of diseased plants (non-emergence + blackleg) using the GENSTAT programme [Payne *et al.*, 1987].

Tuber tissue resistance

Tuber tissue resistance was determined with the slice inoculation method as originally described by Lapwood *et al.*, [1984]. Tuber material from cold storage was either left untreated, presprouted or planted in the field for one to five weeks in order to study the effect of sprouting and development under field conditions. Presprouting was carried out by placing tubers in diffuse daylight for 8 weeks during March to May 1992 at an ambient temperature of approximately 15 °C, according to seed producers' practice. In 1993, tubers were planted in a field with sandy soil on May 26, without prior presprouting treatment. Three plots were planted, each consisting of one row of 25 tubers per cultivar in randomized positions. Plots were harvested in random order, and one at a time, 7, 21 and 35 days after planting. Mother tubers were separated from the sprouts, surface sterilized for 10 min in 1% hypochlorite, rinsed with tap water, dried overnight at room temperature and then used for inoculation. Tubers that were not planted and used as controls when studying the effect of development under field conditions were also surface sterilized.

For inoculation and incubation of tuber tissue, 1 cm thick slices were cut from seed tubers and incubated overnight at 15 °C. One slice per tuber was used for inoculation with *Eca* and another for inoculation with *Ech*. One fresh wound per slice was made by pressing a steel rod 4 mm deep and 5 mm diameter in the medullary tissue just near the pith, in which 20 µl of bacterial suspension was pipetted. Slices were placed at randomized positions in trays measuring 60 × 40 × 6 cm with a perforated bottom that were piled in airtight containers of 65 × 45 × 65 cm. These containers were placed at 20 °C in a growth chamber and flushed with 500 ml min^{-1} air or nitrogen, or mixtures of nitrogen and oxygen, for 5 or 3 days to create different incubation conditions. Ten slices were used for each combination of cultivar, *Erwinia* spp. and incubation condition. After incubation, rotted tissue was carefully removed and the diameter of the lesion measured in two directions at right angles. Experimental layout and analysis of mean lesion diameters were as described by Allefs *et al.*, 1995.

Nine experiments were carried out for studying tuber tissue resistance. Experimental details of these experiments which were numbered from 1 to 9, are presented in Table 1. The results from

Table 1. Summary of the 9 experiments that were carried out for studying tuber tissue resistance. For each of the experiments the year of harvest (yh) of tuber material is given, the month of inoculation and the treatments carried out. In all experiments both *Eca* and *Ech* were used for inoculation. The duration of incubation was 5 days except for the nitrogen treatment in experiments 6 to 9 which was 3 days

Exp.	yh	Date of inoculat.	Treatments
1	1990	Jun '91	incubation in air at 20 °C
2	1991	Mar '92	incubation at 20 °C in air or 2% oxygen
3	1991	Mar '92	incubation at 20 °C in air or 5% oxygen
4	1991	Mar '92	incubation at 20 °C in air or nitrogen
5	1991	May '92	as 4, but tubers presprouted
6	1992	May '93	incubation at 20 °C in air or nitrogen
7	1992	Jun '93	as 4, but tubers harvested 1 week after planting
8	1992	Jun '93	as 4, but tubers harvested 3 weeks after planting
9	1992	Jul '93	as 4, but tubers harvested 5 weeks after planting

experiments 1 to 4 have been described previously [Allefs *et al.*, 1995].

Mother tuber decay in the field

In 1993, tubers of the 12 cultivars were vacuum infiltrated with water, *Eca* or *Ech*, planted at the Wag location and harvested nine times during the growing season in order to follow the process of mother tuber decay. A split plot design was used with three replicates which consisted of three randomized plots, one for each infiltration treatment. Plots consisted of nine randomized subplots, one for each harvest date. Subplots consisted of 12 randomized rows of nine plants, one for each cultivar.

Vacuum infiltration was carried out as described, except that in this case all tubers to be used for one infiltration treatment were divided between four randomized portions which were treated one by one in the same bacterial suspension or aliquot of water (controls). Tubers were stored at 4 °C for 5 (*Ech*, controls) or 6 (*Eca*) days and planted on the 27th of April. No overhead irrigation was applied. Subplots were harvested 4, 5, 6, 7, 9, 11, 13, 17 and 19 weeks after planting. Plants were carefully lifted and the condition of the mother tuber examined. Rotting mother tubers were bisected and the amount of rot estimated. The number of healthy stems and stems showing blackleg lesions of plants with a rotting or vanished mother tuber was counted at harvest dates 1 to 7. Mother tuber decay was calculated as the mean percentage of rot per cultivar within subplots. For each combination of replicate and cultivar, a three parameter ordinary logistic curve was fitted, relating increase of mother tuber decay with time after planting. Eighteen out of 972 data points, with an exceptional effect on the percentage of fit were omitted. The time after planting in days at which, on average, 50 percent of the mother tuber was rotted, further referred to as R50-value, was calculated and its variance analyzed by ANOVA.

Results

Resistance to blackleg

Incidence of non-emergence in control treatments was low except at NOP in 1992 (Table 2). As the experiments proceeded, on average only 0.3% of the control plants developed blackleg. Non-emergence in the controls, especially at NOP in 1992, is therefore thought not to be caused by *Erwinia* but by other unidentified biotic or abiotic factors. Consequently, disease incidence, which includes non-emergence, was corrected for occurrence of non-emergence in the appropriate controls (Table 2). A clear effect of infiltration with *Erwinia* on non-emergence was only found for *Eca* in 1991 when percentages of some cultivars were much higher than in the controls (Table 2).

Analysis of data from either combination of year and location concerning disease incidence after infiltration with *Eca* or *Ech*, resulted in com-

Table 2. Percentage of non-emergence, averaged across cultivars and maximum values. Minimum values were 0 for all combinations of location, year and treatment

Treatm.	Wag 1991		NOP 1991		Wag 1992		NOP 1992	
	mean	max.	mean	max.	mean	max.	mean	max.
Water	0.5	1.6	0.4	1.6	0.7	3.1	7.4	25.0
Eca	6.4	32.8	6.5	18.7	1.1	4.7	7.2	18.7
Ech	0.4	1.6	1.2	6.4	1.6	9.4	8.6	29.7

parable experimental errors (not shown) indicating that combined analysis of these data was allowed. Thus, significant ($P < 0.001$) year × location × cultivar and treatment × cultivar interactions were found. Also a significant ($P < 0.05$) year × treatment × cultivar interaction was found but its effect was less pronounced. In spite of these interactions, disease incidence of 1991 and 1992, when averaged across locations, was well correlated both after infiltration with *Eca* ($r = 0.85$, $n = 12$) and *Ech* ($r = 0.88$). Disease incidence is therefore presented as a mean across both years (Table 3). Cultivars differed significantly ($P < 0.01$) for disease incidence. Disease incidence caused by *Ech*, averaged across cultivars, was equal to the incidence caused by *Eca* at both locations (Table 3) albeit that symptom expression was slightly different: *Ech* caused less non-emergence in 1991 (Table 2), less stunting and more wilting and vascular browning (not shown).

Table 3. Disease incidence (percentage non-emergence + percentage of plants with at least 1 stem with blackleg associated symptoms). Values are means across 1991 and 1992

Cultivar	Wag		NOP	
	Eca	*Ech*	*Eca*	*Ech*
Agria	25.8	39.8	44.5	43.7
Alcmaria	62.5	38.1	51.6	28.9
Amazone	50.0	48.4	43.0	44.5
Arinda	4.7	7.1	12.5	32.0
Bintje	15.6	26.6	17.2	29.7
Désirée	4.7	25.0	14.1	19.5
Hertha	8.9	16.4	18.0	18.9
Karnico	8.6	0.8	8.6	0.0
Kondor	19.5	41.4	40.6	78.1
Morene	19.5	34.4	18.7	26.6
Producent	1.6	1.6	3.1	3.9
Venouska	45.3	33.6	50.0	50.0
mean	22.2	26.1	26.8	31.3

LSD ($P < 0.01$) = 15.3 for cultivar × location × inoculum means; 4.7 for location × inoculum means.

Tuber tissue resistance

Tuber tissue resistance of the cultivars, expressed as the diameter of rotted tissue, was determined in 1991 and 1992 in experiments 1 to 4 (Table 1). Typical results are presented in Table 4, which summarizes tuber tissue resistance after inoculation with *Eca* and *Ech* in experiment 4. Results of experiments 1 to 4, which were all carried out in spring with seed tubers from cold storage, were related to disease incidence (blackleg associated symptoms including non-emergence) as found after vacuum infiltration and planting in the field (Table 5). Although comparisons were made between results of corresponding years i.e. for tubers from the same source, correlation coefficients were low and if found significant ($P < 0.05$), only for one location.

For the experiment carried out to study the effect of presprouting of seed potatoes on tuber tissue resistance (experiment 5, Table 1), it was found that mean diameters of rot across cultivars were 12.3 and 12.6 mm for *Eca* and *Ech* respectively after incubation in air and 21.5 and 24.2 mm after incubation in nitrogen (LSD ($P < 0.01$) = 1.2), which were comparable to mean diameters of rot in experiment 4 (Table 4). Correlation coefficients for comparisons of tuber tissue resistance between both experiments were 0.82 (*Eca*, $r = 12$) and 0.54 (*Ech*) after incubation in air and 0.71 (*Eca*) and 0.81 (*Ech*) after incubation in nitrogen.

Four experiments were carried out in sequence to study the effect of sprout development and growth after planting in the field on tuber tissue resistance (experiments 6 to 9, Table 1). Correla-

Table 4. Summary of the results from experiment 4. Mean diameter of rotted tuber slice tissue (mm), after inoculation with *Eca* or *Ech* and subsequent incubation in air or nitrogen for 5 days at 20 °C

	Incubation condition			
	Air		Nitrogen	
Cultivar	*Eca*	*Ech*	*Eca*	*Ech*
Agria	17.1	11.7	23.4	24.2
Alcmaria	15.9	12.1	20.8	21.8
Amazone	12.1	11.2	13.5	17.1
Arinda	12.6	11.5	20.9	22.6
Bintje	15.7	11.1	20.7	22.2
Désirée	8.6	10.5	18.3	20.8
Hertha	14.3	11.9	15.5	15.0
Karnico	8.4	10.2	18.6	19.4
Kondor	13.5	11.5	25.1	25.6
Morene	12.4	10.2	18.9	19.7
Producent	8.9	9.6	17.3	17.9
Venouska	12.0	11.1	17.9	20.5
mean	12.6	11.1	19.2	20.6

LSD ($P < 0.01$) = 2.3 for cultivar × incubation condition × inoculum means; 0.8 for incubation condition × inoculum means.

tion coefficients for comparisons of tuber tissue resistance between seed tubers from storage and seed tubers that were harvested 1, 3 or 5 weeks after planting are presented in Table 6. Correlation coefficients between resistance of stored and planted tubers decreased rapidly with time after planting, especially after incubation in nitrogen. Five weeks after planting, a significant ($P < 0.05$) correlation was only found for the combination of *Ech* and incubation in air.

Mother tuber decay in the field

Mother tuber decay of seed tubers infiltrated with water, *Eca* or *Ech* was followed during the growing season of 1993. The mean percentage rot of seed tubers that were infiltrated with water increased during the experiment and varied from 59% (cultivar Karnico) to 100% (7 cultivars) at the last harvest date. None of these plants showed stems with blackleg or associated symptoms, indicating that rotting of water infiltrated mother tubers was caused by other microorganisms than *Erwinia*.

Cultivars differed significantly ($P < 0.01$) for the time after planting at which, on average, 50% of the mother tuber was rotted after infiltration with either water, *Eca* or *Ech*. These so called R50 values are presented in Table 7. Mean R50 values across cultivars were significantly ($P < 0.01$) lower after infiltration with *Erwinia* than after infiltration with water, indicating that the *Erwinia* treatment enhanced mother tuber decay. The rate of this enhancement was not equally distributed across cultivars and the cultivar × treatment interaction was found to be highly significant ($P < 0.001$). For example, the cultivars Hertha, Karnico and Producent after *Eca* infiltration, and also Arinda, Bintje and Morene after *Ech* infiltration, showed no significant ($P < 0.01$) decrease of R50 values as compared to the controls, in contrast to the other cultivars.

Table 5. Correlation coefficients for comparisons between blackleg resistance (% of plants with blackleg associated symptoms including non-emergence) and tuber tissue resistance (mean diameter of rot) of stored tubers incubated at 20 °C and several oxygen concentrations. Comparisons refer to experiments of corresponding years and inoculum

Blackleg resistance		Tuber tissue resistance				
inoc.	loc.	1991 air	1992			
			air[1]	5% O_2	2% O_2	nitrogen
Eca	Wag	0.37	0.25	–0.40	–0.35	–0.19
Eca	NOP	0.35	0.59	–0.19	0.07	0.29
Ech	Wag	0.19	0.31	–0.64	0.06	0.01
Ech	NOP	0.20	0.56	–0.22	0.65	0.66

[1] Mean cultivar rot across experiments 2, 3 and 4 were used for calculations.
Significance levels: $r = 0.58$ ($P < 0.05$) and $r = 0.71$ ($P < 0.01$).

Table 6. Correlation coefficients for comparisons of mean cultivar rot between stored tubers and tubers that were harvested 1, 3 or 5 weeks after planting in the field

Time after planting (weeks)	Incubation condition			
	Air		Nitrogen	
	Eca	*Ech*	*Eca*	*Ech*
1	0.89	0.81	0.92	0.86
3	0.73	0.76	0.74	0.34
5	0.46	0.67	–0.16	–0.22

Significance levels: r = 0.58 (P < 0.05) and r = 0.71 (P < 0.01).

The different behaviour of the cultivars is illustrated in Figure 1 which shows the relation between mean percentage rot of mother tubers and time after planting for 4 contrasting cultivars. The fitted logistic curves from Agria and Hertha show that these cultivars had comparable R50 values after water infiltration while only Agria showed a

Table 7. R50 values; mean time after planting (days) at which average mother tuber decay was 50 percent. R50 values relative to the controls are given in parenthesis

Cultivar	Infiltration treatment		
	Water	*Eca*	*Ech*
Agria	85	43 (0.50)	62 (0.74)
Alcmaria	75	45 (0.60)	57 (0.76)
Amazone	93	47 (0.50)	74 (0.80)
Arinda	66	48 (0.73)	69 (1.04)
Bintje	62	43 (0.71)	57 (0.92)
Désirée	94	56 (0.60)	76 (0.81)
Hertha	86	79 (0.92)	76 (0.88)
Karnico	103	92 (0.91)	96 (0.95)
Kondor	72	45 (0.62)	57 (0.79)
Morene	91	50 (0.55)	90 (0.99)
Producent	97	89 (0.91)	96 (0.98)
Venouska	84	53 (0.63)	70 (0.84)
mean	84	58 (0.68)	73 (0.87)

LSD (P < 0.01) = 12 for cultivar × treatment means; 6 for treatment means.

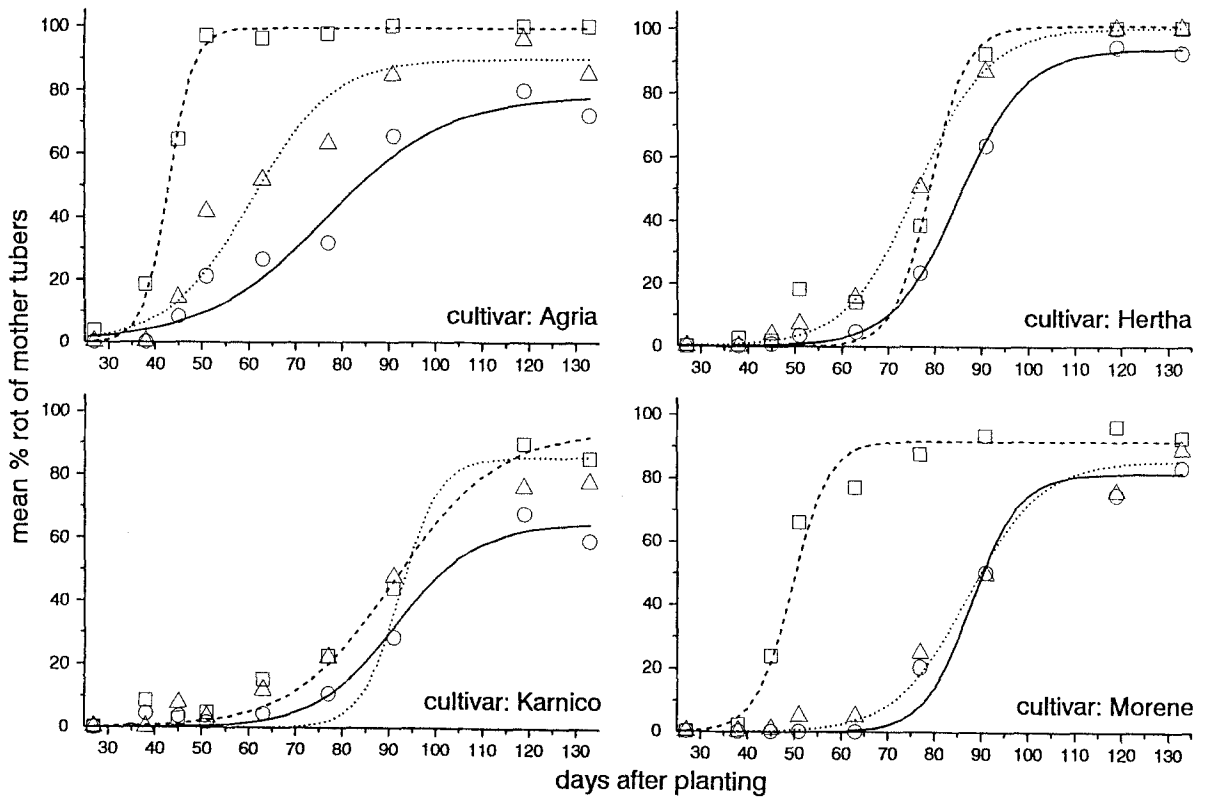

Fig. 1. Mean percentage rot of mother tubers of 4 contrasting cultivars at 9 dates after planting of vacuum infiltrated seed and fitted 3 parameter ordinary logistic curves. Infiltration treatments were water (open circles, ——), *Eca* (squares, ---) and *Ech* (triangles, · · ·).

clear effect of *Erwinia* infiltration. The curves from Karnico illustrate the large R50 values found for this cultivar (Table 7). Another clear cultivar × treatment interaction is seen when curves of Hertha and Morene are compared with only Morene showing an effect of *Eca*.

For each cultivar, the enhancing effect of *Erwinia* on the process of mother tuber decay was expressed as a R50 value relative to the controls (Table 7) and related to the percentage of plants with blackleg associated symptoms averaged across the 6th and 7th harvest dates (Fig. 2). Moderate but significant ($P < 0.01$; *Eca* and $P < 0.05$; *Ech*) negative correlations were found, suggesting that the rate of blackleg development was only partially explained by the effect of *Erwinia* on mother tuber decay. Cultivars Alcmaria and Désirée for example, showed equal relative R50 values for *Eca* but differed strongly for the percentage of blackleg found.

Percentages of plants with blackleg associated symptoms (excluding non-emergence) were 42.1 and 19.9 for *Eca* and *Ech* respectively. Results of the 1993 experiment were in agreement with disease incidence at Wag averaged across 1991 and 1992 ($r = 0.81$, $n = 12$; *Eca* and $r = 0.75$; *Ech*).

Tuber tissue resistance of the cultivars after planting as studied in experiments 6 and 9 was compared to the appropriate relative R50 values of Table 7. For cold stored tubers (experiment 6) only a significant ($P < 0.05$) negative correlation ($r = -0.65$) was found for *Ech* after incubation in air. Tissue resistance of tubers harvested 5 weeks after planting (experiment 9) showed no significant ($P < 0.05$) correlation with relative R50 values at all (data not shown). This was not caused by the results of Karnico and Producent, the only cultivars of which different sources of tubers were used.

Discussion

Resistance to blackleg

Vacuum infiltration is thought to resemble the natural infection via lenticels and small injuries prior to harvest in soil or during post harvest handling more than other methods that have been

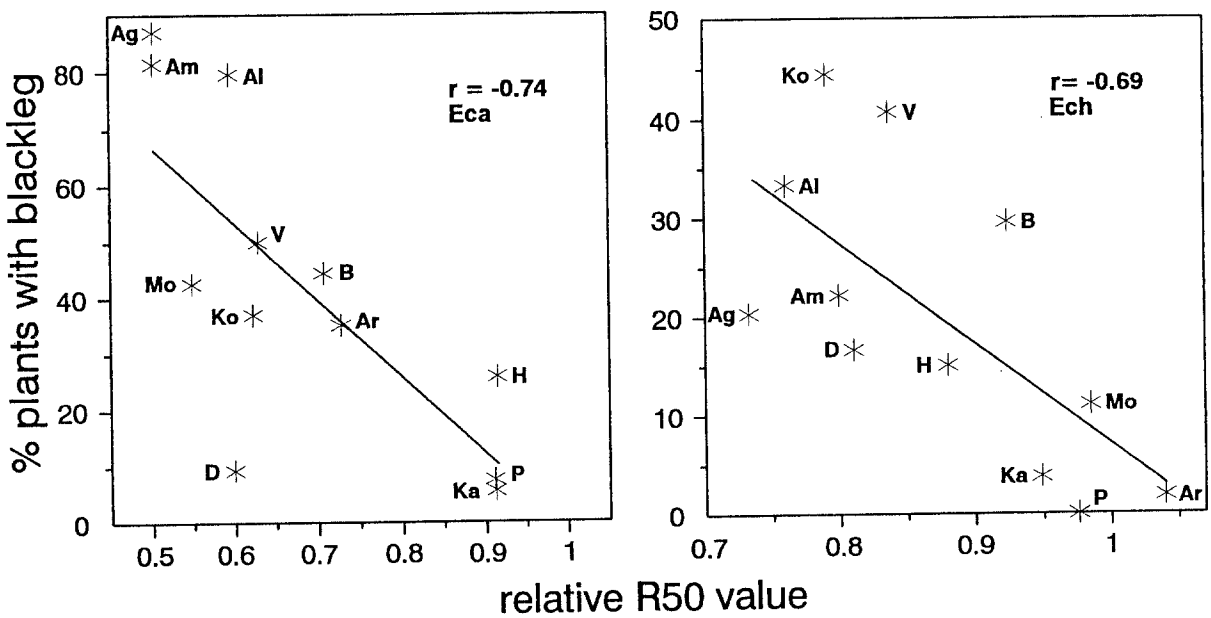

Fig. 2. The relationship between the relative R50 values and percentage of plants with blackleg associated symptoms in the 1993 experiment at Wag for Agria (Ag), Alcmaria (Al), Amazone (Am), Arinda (Ar), Bintje (B), Désirée (D), Hertha (H), Karnico (Ka), Kondor (Ko), Morene (M), Producent (P) and Venouska (V) after infiltration of the seed with *Eca* (left panel) or *Ech* (right panel).

used for field tests [Gans *et al.*, 1991]. Furthermore, Bain and Pérombelon [1988] showed for a set of cultivars that the number of bacteria infiltrated per tuber is comparable.

Correlation of disease incidence data between years was high for both *Eca* and *Ech*, in spite of the significant year × location × cultivar interaction found. Also, results concerning groups of the five most resistant (Arinda, Désirée, Hertha, Karnico and Producent) and most susceptible (Agria, Alcmaria, Amazone, Kondor and Venouska) cultivars were consistent for both locations, except for Arinda and Alcmaria which were relatively susceptible and resistant respectively to *Ech* at NOP. Although the importance of *Ech* as causal pathogen of blackleg in temperate regions relative to the importance of *Eca* is not known, results indicate that screening for blackleg resistance in the field should preferably be carried out at different locations with both *Eca* and *Ech*.

Tuber tissue resistance

It has been shown in a number of studies that the slice inoculation method as originally described by Lapwood *et al.* [1984] is suitable for determination of tuber tissue resistance and that the results obtained, in terms of resistance of clones relative to others, are hardly affected by variation in experimental conditions [Lapwood and Read, 1985; Wastie *et al.*, 1988], although considerable interaction effects of *Erwinia* (sub-)species and oxygen concentration during incubation have been found [Allefs *et al.*, 1995]. However, results from screening for tuber tissue resistance had no predictive value when correlated to results from screening for resistance to blackleg in the field, regardless of the oxygen level during incubation and in spite of the fact that results were compared for experiments in which the same source of tubers had been used (Table 5). Results from correlation studies between tuber resistance and resistance to blackleg in the field have not been described before, although some groups have reported on the relation between tuber resistance and blackleg of glasshouse grown plants. Thus Zadina and Dobiáš [1976] found a correlation coefficient of 0.65 after screening 198 cultivars for resistance to *Eca*, in contrast to the work by Munzert and Hunnius [1980] and Hidalgo and Echandi [1982] who found no correlation for *Eca* and *Ech* respectively. The absence of a correlation can partly be explained by changes in relative tuber tissue resistance after planting in the field (Table 6) which was in contrast to the good reproducibility found for tuber tissue resistance when using tubers stored for various lengths of time, especially after inoculation with *Eca* [Allefs *et al.*, 1995]. Bain and Pérombelon [1988] tested mother tubers for resistance to *Eca* after a one month period of growth in the glasshouse in pots containing field soil, and they too did not find a correlation with results from tests for which stored tubers had been used. Results from experiment 5 show that presprouting of tubers did not markedly affect the ranking of the cultivars for resistance.

Tuber tissue resistance has been determined in air and nitrogen and partly also at intermediate oxygen concentrations. In a well structured and drained soil, oxygen diffuses sufficiently through pores and concentrations in the soil do not differ from the above ground situation. After heavy rainfall or excessive irrigation however, pores become filled with water and dissolved oxygen is soon depleted by plant and microbial respiration [see Drew, 1990]. Disease development is often associated with such conditions [Pérombelon and Kelman, 1980; Weber, 1990; Pérombelon, 1992] and therefore, resistance of seed potatoes under anaerobic conditions is expected to be of importance, probably in interaction with moist conditions as such, since resistance of stored tubers is reduced by high water content [Pérombelon and Lowe, 1975; Weber and Jansen, 1984; Weber, 1988]. In this study, no indication was found that anaerobic resistance of seed tubers is more effective for the control of blackleg than aerobic resistance.

Mother tuber decay in the field

Infiltration of seed potatoes with *Erwinia* enhanced the natural process of mother tuber decay. The rate at which this occurred was dependent on the cultivar, *Erwinia* spp. and their interaction effects (Table 7, Fig. 1).

A significant but relatively small correlation was found between the percentage of blackleg and mother tuber decay expressed as a R50 value relative to the controls (Fig. 2). This indicates that

there is a role of the mother tuber in blackleg development, but that other factors such as the rate to which the rotting process of the mother tuber proceeds into the stem [Munzert and Hunnius, 1980] and resistance of the stem tissue itself [Pérombelon, 1988], might also be important. Since no correlation was found between relative R50 values of mother tubers in the field and tuber tissue resistance, even five weeks after planting, it will be very difficult, if not impossible, to select for clones with high mother tuber resistance by using the slice inoculation method. Tuber tissue resistance of mother tubers which had been grown in the field for more than five weeks could not be determined due to the start of pathogen independent rotting and extensive cavities forming in the medullar tissue. However, as can be seen in Figure 1, the effect of *Erwinia* on mother tuber decay took place in the period beyond five weeks after planting.

Conclusions

Selection for resistance to blackleg in the field by screening stored seed tubers for tuber tissue resistance with the slice inoculation method as originally described by Lapwood *et al.* [1984], either under aerobic or anaerobic conditions, seems not possible. It is not likely that the method can be adapted for this purpose since it was shown that tuber tissue resistance changes soon after planting in field. Also, tuber tissue resistance determined on field planted and subsequently lifted tubers did not show any correlation with the process of mother tuber decay in the field. Mother tuber decay seems to play a significant role in partial resistance to blackleg but other, as yet unidentified factors, are thought to be as important.

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