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Methods of assessing potato tubers for resistance to bacterial soft rot

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Summary

The susceptibility to soft rot caused by *Erwinia carotovora* subsp. *atroseptica* of five potato cultivars and potato clones from the potato breeding programme at Jõgeva Plant Breeding Institute was assessed in a laboratory test. Three test methods were used: a single site inoculation, vacuum infiltration and slice inoculation. The severity of the disease varied widely with the different methods, which gave different cultivar rankings.

Introduction

Soft rot, caused by *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye, is one of the most important diseases of the potato crop. There are no practical chemical controls for reducing decay losses (Łojkowska & Kelman, 1989), and reliance must be placed on resistance breeding. Screening for resistance to soft rotting erwinias has methodological problems: many techniques have been used to evaluate resistance to soft rot (Wastie, 1987), but rank orders do not reflect the behaviour of varieties in practical conditions (Tzeng et al., 1990).

There are relatively few reports comparing results obtained with different methods (Dobias, 1973; Komorowska-Jedrys, 1975; Lapwood et al., 1984; Bain & Pérombelon, 1988). The ranking of the resistance of tubers of different cultivars to soft rot differed in different screening procedures (Tzeng et al., 1990), although the same test gave similar results on different occasions (Wastie et al., 1988).

This paper reports the results of a series of trials comparing the extent of decay after three different inoculation methods.

Materials and methods

The experiments were carried out at the Jõgeva Plant Breeding Institute in 1989 with potato cultivars Eba, Sulev, Vigri and with advanced clones R 21-80 and R 33-82. The tests were repeated eight times during the storage period from September to March. Potato tubers were grown on a sandy clay loam soil and stored at 2 – 7 °C in the dark until required. Healthy undamaged tubers were washed and dried before inoculation but not surface sterilized. There were usually nine tubers or slices of each cultivar in an experiment. Inoculated tubers were dipped in distilled water and placed in closed polyethylene bags containing a few ml of distilled water to achieve anae-

robic conditions. Inoculated slices were incubated on wet filter paper in closed boxes. Inoculated tuber samples were incubated at 25 °C for 3 days before being assessed.

The inoculum of *Erwinia carotovora* subsp. *atroseptica* was a suspension of 10^8 bacteria/ml of several isolates prepared from cultures grown for 24 h on nutrient agar slopes at 25 °C.

Vacuum infiltration. Tubers were immersed in the bacterial suspension and held under vacuum at -80 KPa for 1 min (McGuire & Kelman, 1984). After incubation each tuber was weighed before and after washing away the rotted tissue. The extent of rotting was recorded as the percentage of rotted tissue by weight.

Single site inoculation. This was a modification of the method of Bourne et al. (1981). A hole 10 mm deep and 6 mm wide was made with a sterilized corkborer midway between the rose and the heel of the tuber. Into the hole was pipetted 0.1 ml of the suspension and the hole was sealed with a coverslip. Each tuber was weighed before and after washing away the rotted tissue.

Tuber slice inoculation. This was the method described by Lapwood et al. (1984). A 12 mm thick slice was cut from the centre of each tuber. Two holes 3 – 4 mm deep and 6 mm diameter were made just within the vascular ring with a sterilized corkborer. The wounds were allowed to heal for 6 h before inoculation with 0.02 ml of bacterial suspension. The diameter (mm) of the rot was estimated from two measurements (x and y) made at right angles on the surface of each slice. The mean advance from the edge of the inoculation hole (A) was calculated from: $A = (x + y - 12)/4$.

The 6 h interval between wounding and inoculation was selected because Doke (1982) reported that there were significant biochemical changes in resistant cultivars 6 h after wounding. Some cultivars also begin cork formation within 6 h of wounding (Lyon, 1989).

Because susceptibility to soft rot was measured in different units the results were subjected to Kruskal-Wallis one way analysis of variance.

Results

Resistance to soft rot varied between the different methods (Table 1). Thus cv. Eba evaluated by vacuum infiltration and by the single site method showed more resistance than in the tuber slice method. Advanced clone R 33-82 was more resistant in the tuber slice test than in the other tests (Fig. 1).

The rank order of the cultivars differed with the test method. Thus cv. Vigri was more resistant than clones R 21-80 and R 33-82 in the vacuum infiltration test, more susceptible in the tuber slice test and of similar susceptibility in the single site test (Fig. 2).

Discussion

Variation in rank order between test methods was also reported by Dobias (1973), Lapwood et al. (1984) and Bain & Pérombelon (1988). Each method assesses different components of general resistance.

Vacuum infiltration allows bacterial cells to penetrate the lenticels. This method

Table 1. The susceptibility of potato tubers and tuber slices of different cultivars to soft rot, assessed by vacuum infiltration, single site inoculation and slice inoculation method at different times.

Data	Eba	Sulev	Vigri	R 21-80	R 33-82	LSD* (0.05)
Vacuum infiltration (% rotted tissue)						
6 Sep '89	5.7	12.1	23.2	30.9	24.3	10.2
15 Sep '89	16.0	17.5	22.9	18.3	33.0	10.3
3 Oct '89	11.0	13.0	10.6	36.4	31.8	9.7
17 Oct '89	17.3	8.8	7.1	28.1	18.4	11.3
17 Nov '89	8.4	11.6	9.6	23.1	30.4	11.2
30 Jan '90	16.0	21.6	7.2	19.6	27.6	14.6
13 Feb '90	14.3	7.4	11.8	13.2	27.4	15.7
15 Mar '90	9.7	17.7	14.4	23.9	14.1	10.2
Single site method (g rotted tissue)						
6 Sep '89	11.3	5.2	16.5	11.8	9.8	6.7
15 Sep '89	30.4	11.8	28.3	14.9	21.6	9.9
3 Oct '89	6.7	8.2	23.2	5.7	10.8	7.4
17 Oct '89	15.0	10.3	9.5	10.2	18.2	8.2
17 Nov '89	17.1	16.5	21.1	21.0	21.6	ns
30 Jan '90	9.8	8.9	16.7	15.7	19.7	10.9
13 Feb '90	12.8	8.9	12.8	10.8	13.8	ns
15 Mar '90	15.7	17.7	21.6	18.7	24.6	ns
Slice method (mm rot advance)						
6 Sep '89	5.8	2.9	5.6	4.8	2.4	1.8
15 Sep '89	5.8	0.9	5.8	4.8	5.1	1.9
3 Oct '89	3.9	2.6	5.5	2.4	5.1	3.0
17 Oct '89	5.4	3.2	3.4	2.5	2.5	1.5
17 Nov '89	5.2	2.8	3.4	1.4	1.7	1.7
30 Jan '90	5.4	2.4	2.1	1.8	2.6	1.9
13 Feb '90	4.3	1.5	0.9	0.6	1.8	1.1
15 Mar '90	4.9	0.9	1.4	1.4	1.9	1.3

* ns = not significant at $P < 0.05$.

involves passive mechanisms against penetration in tuber surface and mimics the condition of tubers in waterlogged soil and wet tubers in storage (Tzeng et al., 1990).

The single site method, done under anaerobic conditions, ensures a progressive rot but prevents the normal wound reaction of host tissue and favours the growth of the bacterium. The test measures the suitability of the tissue as a food base for the pathogen (Lapwood et al., 1984) and mimics the anaerobic conditions under which wounded tubers occur in storage or in transit.

The slice test gives some measure of the reaction of the cultivar to a bacterial challenge at a wound and of its capacity for and speed of wound-healing (Lapwood et al., 1984). The method reproduces the aerobic conditions under which wounded tubers are placed after harvest.

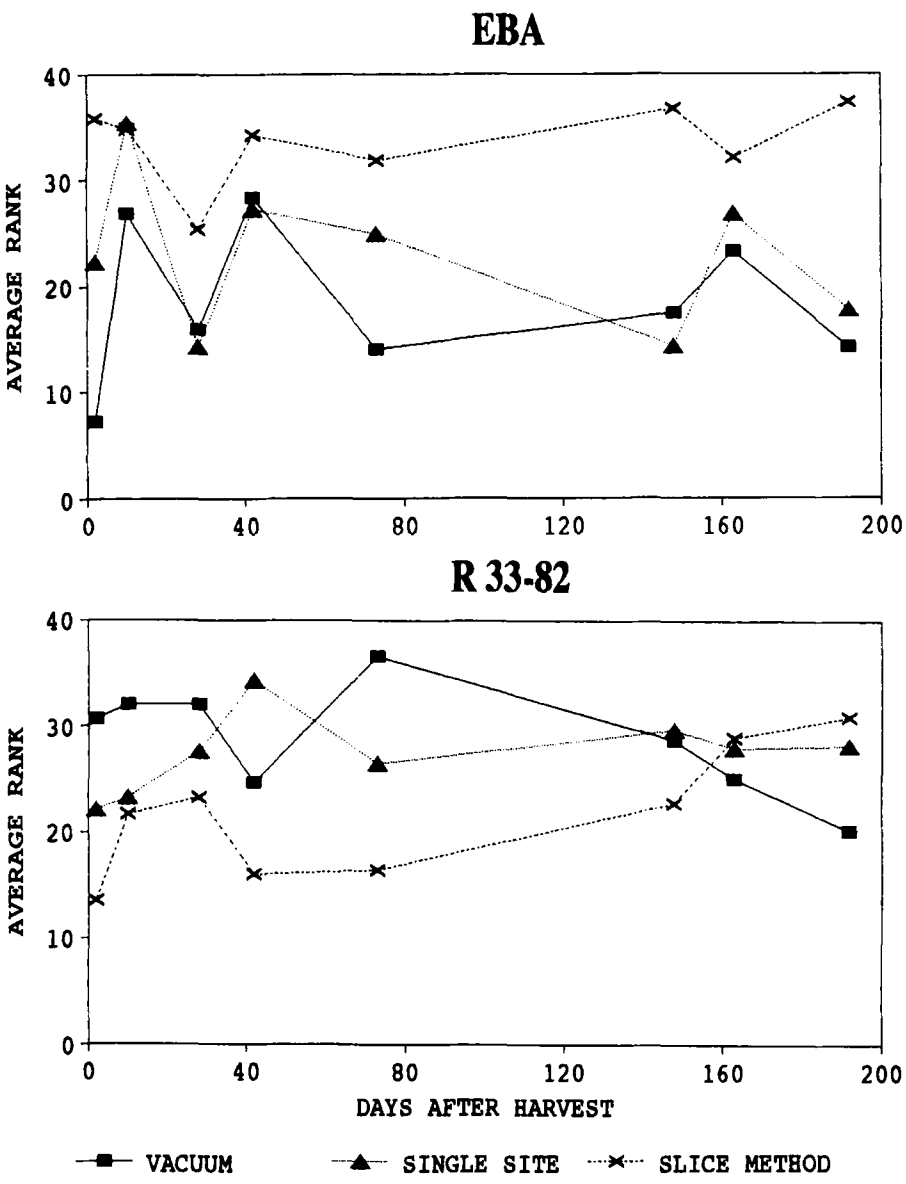

Fig. 1. The resistance of potato tubers of cv. Eba and clone R 33-82 assessed by vacuum infiltration, by single site inoculation and by the slice test. Cultivar resistance is shown as the average rank by Kruskal-Wallis one way analysis.

Resistance to infection and rotting is due to a combination of factors, and pathogenicity is the result of several determinants interacting with each other (Pérombelon, 1987). Rotting is influenced by many environmental and cultural factors

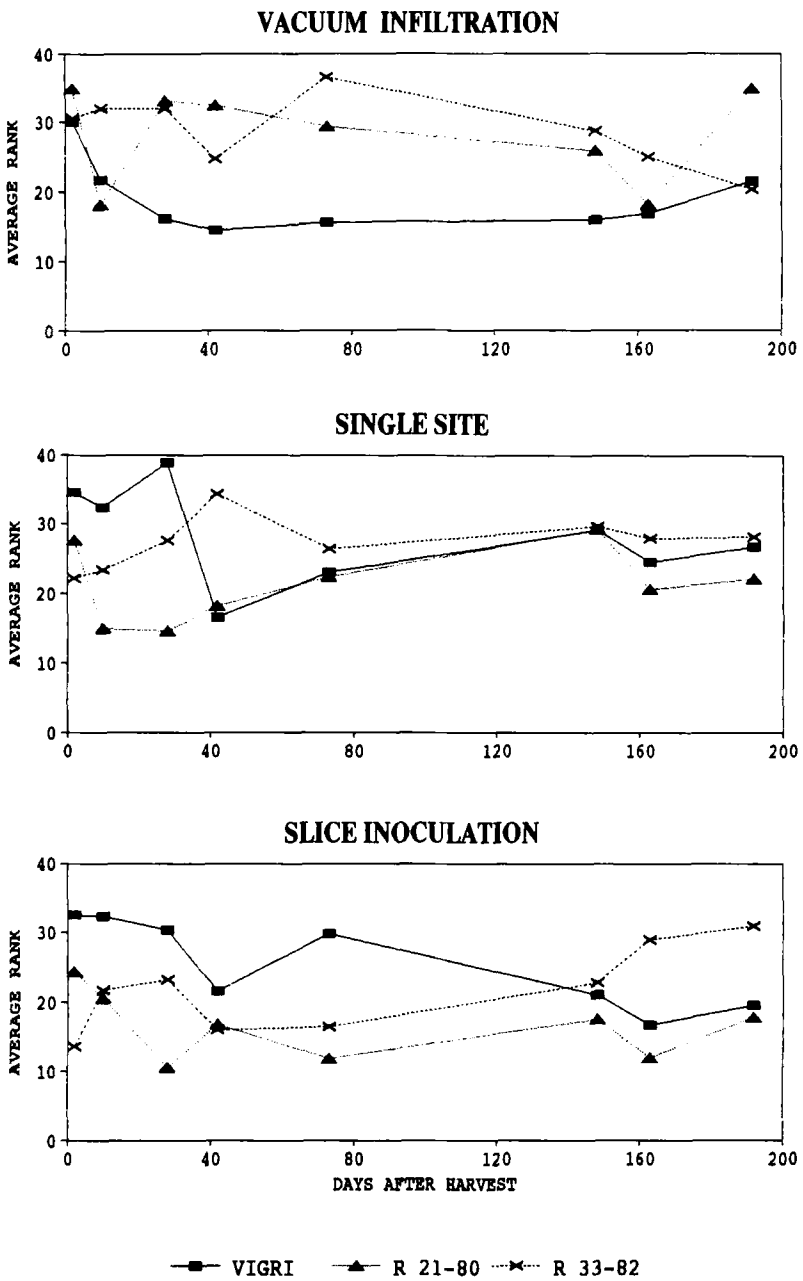

Fig. 2. The resistance of potato tubers of cv. Vigri and clones R 21-80 and R 33-82 assessed by vacuum infiltration, single site and slice inoculation methods. Cultivar resistance is shown as the average rank by Kruskal-Wallis one way analysis.

during growth, harvesting, storage and shipping that may affect resistance (Bain & Pérombelon, 1988). Difficulties exist in establishing which factors are the most important under field conditions.

In storage at Jõgeva cv. Sulev has been the most resistant to soft rot, followed by clones R 21-80 and R 33-82. Cv. Eba has been of medium resistance and cv. Vigri the most susceptible. In my experiments cv. Sulev was resistant in all the different test methods. No single method is appropriate for measuring soft rot resistance, for each approximates to a different set of storage and handling conditions. It is suggested that several methods be used to derive a composite picture for each test cultivar.

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