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Detection of *Erwinia carotovora* var. *atroseptica* in potato tubers with immunofluorescence following induction of decay

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Summary

The simplest method of inducing tubers to decay in order to detect contamination by the blackleg organism, *Erwinia carotovora* var. *atroseptica*, was to wound them and place them in a mist chamber for four days. Incubating the decayed tubers in air for a further two days improved detection of the blackleg organism by immunofluorescence, which was a more sensitive test than the double diffusion method.

Introduction

Since seed potato tubers are the major source of inoculum for blackleg (Perombelon, 1974), those free of *Erwinia carotovora* var. *atroseptica* (van Hall) Dye are the most likely to give a blackleg-free crop. Several methods have been reported for determining blackleg contamination on potato tubers. All involve inducing tubers to decay and subsequent isolation or serological detection of bacteria (De Boer & Kelman, 1975; Perombelon, 1972; Vruggink & Maas Geesteranus, 1975). A more efficient method was desired for large-scale testing of seed potatoes, and in this study the efficacy of an immunofluorescence method (Allan & Kelman, 1977) was compared with the double diffusion method (Vruggink & Maas Geesteranus, 1975) for detection of *E. carotovora* var. *atroseptica* in potato tubers induced to decay. Several methods for inducing decay were also compared.

Materials and methods

Serological techniques

Antiserum was prepared in rabbits against an isolate of *E. carotovora* var. *atroseptica* (Isolate R 10) and absorbed with a cross-reacting strain of *E. carotovora* var.

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carotovora (Isolate F) as reported previously (Vruggink & Maas Geesteranus, 1975). The antiserum was conjugated with fluorescein isothiocyanate by the method of Allan & Kelman (1977).

All decayed tissue was washed from rotted tubers, filtered through cheese cloth and centrifuged at 400 *g* for 5 min. The pellets were resuspended in 0.5 ml water and either tested in agar double diffusion plates (Vruggink & Maas Geesteranus, 1975), or individual loopfuls were heat-fixed on a slide and stained with the fluorescein-antibody stain (FAS). Slides were observed with dark field illumination at × 400 under a fluorescence microscope with a heat filter, a BG 12 excitation filter and a K460 barrier filter.

Tuber injury and infiltration

Initially toothpicks were used to puncture 5 lenticels per tuber to a depth of about 10 mm. In later experiments a board with 25 nails, 1.8 mm in diameter, protruding 5 mm beyond the board and spaced 5 mm apart in a square pattern, was employed to injure about 75% of the tuber surfaces. This device, subsequently referred to as the nail board, was dipped in alcohol and briefly flamed after injuring one and before injuring another tuber.

Instead of injuring, some tubers were infiltrated with water by placing them in a container of tap water and subjecting them to a partial vacuum (100 mm Hg) for 15 min.

Conditions for tuber incubation

To induce potato tubers to decay they were either: (1) wrapped individually in moist paper towels and a double layer of plastic film (De Boer & Kelman, 1975); (2) placed between layers of moist paper towels in trays enclosed in plastic bags; (3) placed on wire racks in a mist chamber (Lund & Kelman, 1977); or (4) submerged in bulk in tap water (Vruggink & Maas Geesteranus, 1975). The incubation temperature was 20 C except in the mist chamber where the tap water used for misting was about 16 C.

Results

Detection of the blackleg organism

The double diffusion method detected the blackleg organism in only 10% of the samples containing bacteria staining positive with FAS (Table 1). Although the amount of tuber tissue was not quantified; preparations of decayed tissue that gave a positive result in double diffusion usually had more than 100 FAS-stained cells/microscope field. In 4 of 330 samples, from various experiments in which different methods were used to induce decay, a positive result was obtained with double diffusion when no or very few cells were found in slide preparations stained with FAS. Almost 50% more tubers were found to be positive with immunofluorescence when the decayed tubers were incubated in air for 2 days before making the slide preparations than when tested immediately (Table 2).

Table 1. Number of tubers, out of 500, in which *Erwinia carotovora* var. *atroseptica* was detected by: A, immunofluorescence; and B, double diffusion*.

Tuber lot**	Number of tubers	
	A	B
1	117	11
2	86	3
3	93	2
4	82	7
5	151	36
Total	529	59

* Tubers were induced to decay by submersion in tap water.
** Each tuber lot was grown in a different area of the Netherlands and consisted of three or four of the following cultivars: Bintje, Nicola, Climax, Saskia, Alpha, Element, Kromanta, Ostara, Krostar, Vokal, Desirée, and Jaerla.

Tuber incubation for inducing decay

The blackleg bacterium could be detected in more tubers incubated under water than in those wrapped individually, although decay lesions developed on all tubers in both treatments (Table 2). The possibility of cross-contamination between tubers incubated under water could not be ruled out, however. To decrease the work involved in wrapping tubers individually, some were placed between layers of paper towels in trays placed in plastic bags, but they usually did not then decay as readily as when wrapped individually (Table 3). Furthermore, leakage from decayed tubers was shown to cause cross-contamination. Incubating tubers on wire racks in a mist chamber for four days maintained conditions favourable for decay, prevented cross-

Table 2. Percentage of decayed tubers in which *Erwinia carotovora* var. *atroseptica* was detected by immunofluorescence in tissue sampled immediately after anaerobic incubation (A) and after a further 2 days under normal atmospheric conditions (B).

Method for inducing decay	Tubers with *E. carotovora* var. *atroseptica** (%)			
	A		B	
	1976	1977	1976	1977
Lenticels punctured and tubers wrapped individually	10	28	52	80
Tubers submerged in water	32	44	74	96
Mean	28.5		75.5	

* 50 tubers per treatment in 1976 and 25 tubers per treatment in 1977.

Table 3. Number of tubers, out of 25, in which *Erwinia carotovora* var. *atroseptica* was detected by immunofluorescence after inducing decay by several methods. Numbers in brackets indicate the number of tubers which decayed. Prior to incubation the tubers had been injured (inj.) or infiltrated (inf.) or both (inf. + inj.).

Variety	Wrapped individually in moist paper towels and plastic film (20 C)	Between moist paper towels in trays placed in plastic bags (20 C)			In mist chamber (16 C)		
	inj.	inj.	inf.	inf. + inj.	inj.	inf.	inf. + inj.
Climax	12(25)	11(17)	25(25)	16(25)	16(25)	24(24)	12(25)
Climax	–	10(18)	8(23)	-	11(25)	11(24)	
Desiree	25(25)	9(9)	25(25)	22(25)	25(25)	25(25)	24(25)
Ostara	25(25)		10(10)	–	25(25)	15(15)	25(25)
Bintje	24(25)	–	12(12)	–	25(25)	–	–

contamination and permitted equally good detection of the blackleg pathogen when compared with other methods (Table 3).

Regardless of the method of incubation, tubers decayed most readily and uniformly when injured with the nail board before incubation. Infiltration of the tubers with water was also effective in promoting the development of decay but this was not further enhanced by injuring the tubers after infiltration (Table 3).

Discussion

Absorption of the antiserum with a cross-reacting *E. carotovora* var. *carotovora* strain rendered it specific for *E. carotovora* var. *atroseptica*, as reported previously (Vruggink & Maas Geesteranus, 1976). The blackleg organism was detected by immunofluorescence in about 10 times as many tubers as with the double diffusion technique (Table 1). Immunofluorescence had the added advantage that it obviated the need for isolation and biochemical characterization of the bacteria. In those instances where bacteria could not be found with immunoflourescence although a positive result was obtained in double diffusion, could be explained on the assumption that the bacterial cells could have become disrupted in the decayed tissue, while the soluble antigens, utilized in double diffusion, remained intact.

Erwinia carotovora var. *atroseptica* could be detected more readily when the decayed tubers were exposed to normal aerobic conditions before testing than when tested immediately after anaerobic incubation (Table 2). Under anaerobic conditions pectolytic *Clostridium* spp. often contribute to decay (Lund & Nicholls, 1970) and could compete with the blackleg organism; under aerobic conditions the clostridia are inhibited and the blackleg organism is favoured.

Blackleg bacteria in quiescent infections were induced to multiply by depleting the oxygen available to the tubers rendering them susceptible to decay (De Boer & Kel-

man, 1978). Relatively anaerobic conditions were provided during tuber incubation by maintaining a film of moisture on the tuber surface (Burton & Wigginton, 1970; Lund & Kelman, 1977). In addition to low oxygen conditions, injury and water infiltration increased tuber susceptibility and favoured initiation of bacterial soft rot.

The effect of incubation temperature was not studied, but tubers decayed readily at both 16 and 20 C. Incubation at 16 C may be found to facilitate detection since *E. carotovora* var. *atroseptica* tends to predominate more at 16 C than at 20 C (M.C. M. Pérombelon, personal communication).

The simplest and most efficient method for detecting contamination by the blackleg bacterium was to injure tubers with the nail board, place them in a mist chamber for four days and test the decayed tissue with immunofluorescence after an additional two days of aerobic incubation. The percentage of tubers in which the blackleg organism could be detected, however, varied between samples from a single potato lot tested at different times by the same procedure, possibly due to the difficulty in obtaining a random sample. The immunofluorescence technique was useful for large scale testing in our laboratory and should prove useful in a seed certification programme.

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References

Burton, W. G. & M. J. Wigginton, 1970. The effect of a film of water on the oxygen status of a potato tuber. *Potato Res.* 13 : 180–186.

De Boer, S. H. & A. Kelman, 1975. Evaluation of procedures for detection of pectolytic *Erwinia* spp. on potato tubers. *Am. Potato J.* 52 : 117–123.

De Boer, S. H. & A. Kelman, 1978. Influence of oxygen concentration and storage factors on susceptibility of potato tubers to bacterial soft rot (*Erwinia carotovora*). *Potato Res.* 21 : 65–80.

Lund, B. M. & A. Kelman, 1977. Determination of the potential for development of bacterial soft rot of potatoes. *Am. Potato J.* 54 : 211–225.

Lund, B. M. & J. C. Nicholls, 1970. Factors influencing the soft-rotting of potato tubers by bacteria. *Potato Res.* 13 : 210–214.

Pérombelon, M. C. M., 1972. A reliable and rapid method for detecting contamination of potato tubers by *Erwinia carotovora*. *Pl. Dis. Reptr* 56 : 552–554.

Pérombelon, M. C. M., 1974. The role of the seed tuber in the contamination by *Erwinia carotovora* of potato crops in Scotland. *Potato Res.* 17 : 187–199.

Vruggink, H. & H. P. Maas Geesteranus, 1975. Serological recognition of *Erwinia carotovora* var. *atroseptica*, the causal organism of potato blackleg. *Potato Res.* 18 : 546–555.