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Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967-74

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Zusammenfassung, Résumé p. 17

Summary

Propagation of potato stocks from stem cuttings has produced material almost entirely free from infection with *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica*; during 1967-74, very few infected plants or tubers were found on the nuclear stock farm. When infection occurred, *E. carotovora* var. *carotovora* was the organism most often isolated, whereas in ordinary commercial stocks *E. carotovora* var. *atroseptica* predominates. In 1973 and 1974 dipterous insects caught at a nearby dump of decomposing vegetable matter were contaminated with *E. carotovora* var. *carotovora* several weeks before infection was found on potato stems. Serotypes of *E. carotovora* var. *carotovora* isolated from insects and infected plants were very often identical, providing compelling evidence that the source of the organisms was the dump, from which contaminated insects dispersed and subsequently transferred organisms to the crop. The origin of infections with *E. carotovora* var. *atroseptica* remains unknown.

Introduction

In recent years, research on a number of potato tuber diseases has focused attention on the importance of the mother tuber as the major source of pathogenic micro-organisms attacking both the plants grown from the mother tubers and their progeny. Potato blackleg, caused by *Erwinia carotovora* var. *atroseptica* (subsequently referred to as *E. atroseptica*), and tuber soft rot, which can be caused by both *E. carotovora* var. *carotovora* (subsequently referred to as *E. carotovora*) and *E. atroseptica*, belong to this group of diseases: Graham & Hardie (1971) summarized the evidence that soft rot coliform bacteria are not inhabitants of Scottish soils. Isolations made from blackleg infected stems in Scotland almost always yield *E. atroseptica* (Graham & Dowson, 1960) whereas both *E. carotovora* and *E. atroseptica* were

isolated from all of 48 Scottish commercial tuber stocks, usually in the ratio of 80% *E. atroseptica* to 20% *E. carotovora* (Perombelon, 1973). Much of this infection is latent - tubers show no visible signs of disease throughout the storage period. The important fungal diseases, skin spot and gangrene, also appear to be largely stock-borne. Because the pathogens that cause these diseases are associated with the tuber, new generations of tubers produced by propagation from stem cuttings are much less likely to be infected. However, as soft rot coliform bacteria can spread into potato stems from mother tubers, cuttings must be tested bacteriologically and found free from infection before propagation (Graham & Hardie, 1971). Aiming at a general reduction in the incidence of blackleg, soft rot and other latent tuber diseases in potatoes, the Department of Agriculture and Fisheries for Scotland (DAFS) attempted to obtain nuclear seed stocks from tested stem cuttings using existing virus-free stocks in 1967. The early results were so promising that it was decided to produce all virus-tested nuclear stocks in this way with the eventual objective of replacing all potato stocks in Scotland with material originating from stem cuttings. Nuclear stocks grown from stem cuttings are referred to as VTSC (i.e. derived from virus-tested stem cuttings) and constitute the highest grade in the DAFS Seed Potato Certification Scheme. To avoid re-infection as far as possible the nuclear VTSC stocks have been raised on an upland farm called Ingraston, some 30 km south-west of Edinburgh, in an area where very few other commercial crops of potatoes are grown. Before 1967 Ingraston farm itself had never grown potatoes. Tubers originally derived from stem cuttings are released to specialist VTSC growers for further multiplication who, in turn, pass their produce to growers of lower grades. The background to the VTSC project, the production of stem cuttings and their propagation, methods for isolating and testing for pathogenic organisms, possible transmission of infection by insects and some early experiences relating to re-infection, are discussed by Graham & Hardie (1971).

This paper describes the successful production of VTSC stocks at Ingraston in relation to soft rot coliform bacterial infection. It also discusses recurrence of infection - incidentally widening the general understanding of the epidemiology of soft rot diseases caused by *Erwinia* spp. Bearing in mind the distances separating the Ingraston crop from other commercial crops (usually at least 50 km), the occurrence of infection at Ingraston despite strict hygienic precautions, and reports in the literature of association of insects with soft rot coliforms, led to a bacteriological study of insects in the Ingraston environment. To establish more precisely the identity and thus any epidemiological relationship between soft rot coliforms from sources in and around Ingraston, serotyping was carried out, as is commonly done in studies on the epidemiology of other genera of the Enterobacteriaceae.

Bacteriological and serological methods used in the epidemiological study

Method of isolation and identification of Erwinia spp.

Plant material was suspended in a little sterile water and a loopful plated on Mac-

Table 1. Organisms used to prepare antisera.

Organism[1]	Reference number[2]	Source plant[3]	Country of origin[4]
E. carotovora var. *carotovora*	NCPPB 438	Iris	USA
	NCPPB 671	Carnegiea	USA
	NCPPB 1742	Brassica	Brazil
	NCPPB 547	Persea	Israel
	NCPPB 1745	Brassica	Japan
	NCPPB 312	potato tuber[5]	Denmark
	NCPPB 66	tobacco[6]	Uganda
	NCPPB 550	tobacco	USA
E. carotovora var. *atroseptica*	G 110	potato stem[7]	Scotland

[1] *Erreger - Organisme;* [2] *Bezugsnummer - Numéro de référence de l'antisérum;* [3] *Wirtspflanze - Plante infectante;* [4] *Herkunftsland - Pays d'origine;* [5] *Kartoffelknolle - Tubercule de pomme de terre;* [6] *Tabak - Tabac;* [7] *Kartoffelstengel - Tige de pomme de terre*

Tabelle 1. Erreger, die zur Herstellung der Antiseren verwendet wurden.
Tableau 1. Organismes utilisés pour préparer l'antisérum.

Conkey pectate medium (Stewart, 1962), incubated at 26 °C, and pectolytic colonies characteristic of soft rot coliform bacteria transferred to nutrient agar slopes. Organisms from the slopes were replated on nutrient agar and single colonies transferred to nutrient agar slopes to ensure culture purity. On occasion the MacConkey pectate plates were heavily overgrown with other organisms making it impossible to select discrete colonies. In these circumstances a loopful of colony growth was transferred to a potato tuber slice and incubated on damp filter paper in a Petri dish at 26 ° for 24 h. Uninoculated slices were kept as controls. The inoculated potato slice usually rotted, acting as an enrichment medium for the soft rot bacteria. Isolations were then made from the rotted tissue as before.

When insects were tested for soft rot coliforms, individuals or several insects bulked together were crushed in a drop of sterile water between sterile microscope slides. A large loopful of fluid from the slide was plated on MacConkey pectate; pectolytic colonies were picked from the plates and purified as previously described.

Soft rot coliform organisms were identified to specific and sub-specific level by the methods described by Graham (1972).

Serotyping

Isolates of *E. carotovora* obtained in 1971-74 were serotyped using a range of antisera prepared against the organisms listed in Table 1. Lazar (1972) has shown that there are a large number of serotypes amongst the soft rot coliform group and

Table 2. Serotypes of *E. carotovora* var. *carotovora* found on plant material and insects at Ingraston farm.

Antiserum	Serotype									
	1	2	3	4	5	6	7	8	9	10
NCPPB 438	−	+	+	−	−	−	−	+	−	−
NCPPB 671	−	−	−	−	−	−	+	−	−	−
NCPPB 1742	+	+	−	+	−	−	−	−	−	−
NCPPB 547	−	−	+	−	+	−	−	−	−	−
NCPPB 1745	+	+	−	−	−	−	−	−	+	−
NCPPB 312	−	+	−	−	−	−	−	−	−	+
NCPPB 66	−	−	−	−	−	−	−	−	−	−
NCPPB 550	−	−	−	−	−	−	−	−	−	−
G 110	+	+	−	−	−	−	−	−	+	−

Tabelle 2. Serotypen von *E. carotovora* var. *carotovora*, die an Pflanzen und Insekten in Ingraston gefunden wurden.
Tableau 2. Sérotypes d'*E. carotovora* var. *carotovora* trouvés sur le matériel végétal et les insectes à la ferme d'Ingraston.

most of the antisera were received from Dr Lazar. Antisera against NCPPB 66 and NCPPB 312 were prepared by us according to the method described by Graham (1963). All antisera had titres lying between 1:1000 and 1:2000.

Serotyping was carried out using a simple slide agglutination test. A loopful of a 24-48 h culture grown on nutrient agar was emulsified in a drop of tap water on a slide, 1 drop of antiserum (diluted 1:10 with 0.85% saline) was added and mixed by rocking. Agglutination reactions were read within 2-3 min of adding antiserum.

During the years 1971-74 inclusive, soft rot coliform infections, mainly caused by *E. carotovora* were found on VTSC potato material and from other sources at Ingraston, except in 1972 when infection with *E. atroseptica* predominated in the growing crop. Table 2 gives details of the serotypes of *E. carotovora* found over the period 1971-74. Isolates of *E. atroseptica* were not serotyped.

Although the fact that 2 organisms from different sources prove to be the same serotype does not necessarily imply an origin in common, it is believed that in the rather isolated environment of Ingraston, identity of serotype suggests strongly that the organisms are of common origin.

Epidemiological studies at Ingraston Farm relating mainly to occurrence of Erwinia carotovora var. carotovora on plants, tubers and insects

Investigations were made both during the growing season and on potato tubers in storage; these are described chronologically.

Growing seasons 1967-70

Although mother tubers were known to be the main source of soft rot coliform contamination of daughter tubers, one could not foresee how successful the stem cutting project might be in the short or long term. There were many conceivable ways in which re-infection might occur, such as by contaminated machinery (an obvious example), but also through other avenues so far unproven or unknown. However, every attempt was made to ensure that nuclear stocks were propagated under the best hygienic conditions attainable. Over the period 1967-70 inclusive about 260 000 plants were grown at Ingraston; all were rigorously inspected but only 1 plant (cv. Redskin) was found infected, in this case with *E. atroseptica*. The source of infection was never traced. Eighty-four other samples of suspect plants were examined bacteriologically but found uninfected.

Storage periods 1967-70

Tubers of all stocks stored at Ingraston were carefully inspected throughout the storage periods for soft rot, heel end necrosis or other doubtful symptoms. Out of 1636 stocks 11 contained soft-rotted and other suspect tubers which were subjected to a bacteriological examination. Of these 6 did not yield soft rot coliform bacteria, but 1 stock gave *E. atroseptica* and 4 stocks gave tubers infected with *E. carotovora*.

Growing season 1971

In late August and early September, 21 soft rot infections distributed at random throughout an area containing about 4000 plants were discovered on stems of tested first-year stem cuttings. From the more severe symptoms on two plants with stem lesions it was clear that they had been infected some weeks previously and tubers on these plants showed extensive internal rots caused by organisms identified as *E. atroseptica*. Both plants were next to wheel tracks suggesting infection had been introduced by machinery. A nearby plot, containing plants grown from tubers two years away form the stem cutting stage, contained several blackleg-infected plants which might have been the source. The remaining infections on the stem cuttings were greenish or brownish wet rots either on stems above soil level or at exposed leaf scars, and apparently had been established recently because lesions had not spread extensively. The plants suffered wind damage and because of prolonged wet weather, the haulms were senescent and rotting: the rotting material was attracting a large number of insects including many fruit flies (*Drosophila* sp.) and some wasps (*Vespa* sp.); 12 *Drosophila* , 3 *Vespa* and 5 other unidentified insects were collected and 2 - a *Drosophila* sp. and a *Vespa* sp. - yielded soft rot coliform bacteria. Isolations were made from 21 infected stems; 16 isolates of soft rot coliforms were obtained but were identified only to generic level and were discarded before further tests were made. However, two isolates from the fruit fly and the one from the wasp were identified to specific level and found to be *E. carotovora*. Further isolations were made from first-year stem cutting stems in mid-October after the crop had been lifted. This could be done because diquat had been used as a haulm destroyer,

and as the weather remained wet, it was quite easy to find rotting stems at that time. From 18 stems 18 *E. carotovora* and 2 *E. atroseptica* isolates were obtained, whereas no *Erwinia* spp. were isolated from another 6 stems. This was an unexpected result, because, as mentioned earlier, the organism almost invariably isolated from rotting stems in commercial crops in Scotland is *E. atroseptica*. Pathogenicity tests on potato stems, by the method of Graham & Dowson (1960), showed that 6 isolates from stem cuttings, 1 from *Drosophila* sp. and 1 from *Vespa* sp. did not cause typical blackleg disease, but a localized soft rot, showing that the organisms were pathologically distinct from *E. atroseptica*. The total number of *E. carotovora* isolates collected from the stems and insects was 21, of which 13 were serotyped; 6 stems yielded serotype 1; 3 stems serotype 6; 1 stem serotype 7; 1 *Drosophila* sp., serotypes 6 and 8, and 1 *Vespa* sp., serotype 1. It was concluded that insects in the crop were contaminated with *E. carotovora*, and were probably spreading infection, but it was impossible to say whether the insects had introduced the bacteria, or whether the insects had become contaminated with the organisms while feeding on already infected stems.

Storage period 1971-72

Twenty stocks out of 501 contained tubers affected with soft rot or heel end necrosis; altogether 62 such tubers were found. Seven stocks were infected with *E. carotovora* alone and 8 with *E. carotovora* and *E. atroseptica* together. These results do not correspond with the situation in ordinary commercial stocks, in which infections with *E. atroseptica* predominate. However the amount of disease in the stocks should not be overestimated from these data for only 62 diseased tubers were found out of a total production of some 80 tonnes (although, of course, other tubers could be carrying latent infection). It is also noteworthy that the number of plants constituting a stock grown in earlier years was generally smaller than in 1971 or later years.

Of the isolates of *E. carotovora* 4 were serotyped: 2 were of serotype 1, 1 of serotype 6, and 1 of serotype 7. All these serotypes were found in the growing crop in 1971.

Growing season 1972

Because insects might have been responsible for introducing infection into first year stem cutting material, it was decided to investigate whether insects in the immediate environment of the crop were contaminated with soft rot coliforms. An electrically driven insect trap was made by mounting a 30-cm diameter Vent-Axia fan vertically on a stand 1.5 m high. A muslin bag was attached beneath the fan, protected from wind by a metal cylinder which fitted round the bag and was fixed to the underside of the Vent-Axia mounting. The fan was operated continuously in the crop from mid-June until mid-September. Insects were caught dry in the bag which was changed twice a week; the collections were sorted and individuals identified to the order and sometimes the genus to which they belonged, and tested for soft rot

coliforms. Live insects were also swept from vegetation immediately surrounding the potato field, and taken to the laboratory for plating within 24 h. Additionally, special attention was paid to a turnip crop growing close to the potatoes.

Between 14 June and 14 September 1494 insects comprising 633 Diptera, 216 Coleoptera, 152 Hymenoptera, 253 Hemiptera, 153 *Thrips* spp., 28 Lepidoptera and 49 others were tested. Of these 909 were caught in the trap, 242 were swept from the turnips, and the rest were obtained from other sites around the crop. Only 4 insects, all of which were caught alive in the turnip crop, yielded soft rot coliform bacteria, in every case *E. carotovora*: a hymenopteran caught on 27 July, a dipteran caught on 24 August, another dipteran on 7 September, and a hymenopteran on 14 September.

No soft rot infections of stems similar to those in 1971 occurred on the first-year stem cuttings (though typical blackleg was seen sporadically in plots 2, 3 and 4 years beyond the stem cutting stage and the causal organisms identified as *E. atroseptica*). Furthermore, as the insects carrying the bacteria came from the turnips, these plants were scrutinized carefully. On 31 July, 1 turnip was found with extensive soft rot beginning at the petiole bases; this rot was found to be caused by *E. carotovora*. On 3 August, another similarly infected plant was found, and by the second week in September about a quarter of the crop (some 2000 plants) was affected. Rotting always began on the above ground parts of the plants, suggesting some kind of airborne transmission. Three isolates from insects and 3 from the plants were all found to be serotype 8.

The observations again showed that insects were contaminated with and possibly transmitting soft rot coliforms, but their significance as vectors could not be judged as it was impossible to say whether the insects brought infection into the crop or had become contaminated when feeding on already infected plants.

Live insects always yielded many more bacteria than those from the insect trap; it was concluded that the latter method of collection was unsuitable, probably because the air stream constantly blowing through the bag dried up the insects and apparently killed many of the associated bacteria.

The haulms of the VTSC crop were destroyed with sodium chlorate sprays during August and September, and because of the dry weather the haulm bleached and dried quickly. Eleven samples of stems taken at random from the crop and tested did not yield any soft rot coliforms.

Storage period 1972-73

Three stocks out of 367 contained soft rotted tubers from which *E. carotovora* was obtained. None were serotyped as no *E. carotovora* had been isolated from the 1972 growing crop. However, it was later realized that it would have been of value to know whether the organisms from the tubers were of the same serotype as those found on insects and turnips in the 1972 growing season, but the cultures had been destroyed.

Fig. 1. Site of waste dump where insects were found contaminated with soft rot coliform bacteria in 1973 and 1974. The arrow marked 1973 indicates the precise location where the insects were caught; the arrow marked 1971 shows the area in the field in the background where infected first year-stem cuttings were found in 1971.

Abb. 1. Lage des Abfallhaufens an dem 1973 und 1974 Insekten kontaminiert mit coliformen Nassfäulebakterien gefunden wurden. Der Pfeil, markiert mit 1973, gibt die genaue Stelle an, wo die Insekten gefangen wurden; der andere Pfeil, markiert mit 1971, zeigt im Hintergrund die Stelle des Feldes wo 1971 infizierte Pflanzen aus dem Nachbau der Stengelstecklinge gefunden wurden.

Fig. 1. Emplacement du dépôt de détritus où les insectes ont été trouvés contaminés par les bactéries coliformes de la pourriture molle en 1973 et 1974. La flèche marqué 1973 indique la localisation précise où les insectes ont été capturés; la flèche marquée 1971 montre la partie du champ à l'arrière-plan où des boutures de tige de première année ont été trouvées infectées en 1971.

Growing season 1973

Occurrence of *E. carotovora* infections on stem cuttings and an annual crop, like turnips, on the farm continued to suggest that there might be a source of this organism nearby, from which insects could become contaminated and transmit infection to the crop.

Accordingly, the environment surrounding Ingraston was searched on 24 July, and at any site where dead leaves or other plant debris were found, insects were collected and taken to the laboratory for identification (usually to generic level) and bacteriological examination. One site near a disused railway line consisted of an

area roughly 50 m × 30 m of decomposing vegetable matter, just outside the confines of the farm. Special attention was paid to it, as it was only some 120 m south-west of the place where VTSC stem cuttings were infected in 1971 with *E. carotovora* and 150 m north-west of the 1973 VTSC crop. The site, illustrated in Fig. 1, was heavily overgrown with weeds, but quantities of garden waste had evidently been deposited repeatedly, and 4 potato plants were found growing close together at one place. It was learned later that both farm and garden waste had been deposited there for many years. Sweepings for insects were made in the immediate vicinity of the potato plants; and 15 potato tubers which had already formed on the plants were tested and found infected with *E. carotovora* serotype 1. Insects were again collected on 1 August, 5 September and 16 October for testing for soft rot coliforms, some separately and others bulked according to the genus. From plates on which bulked material had been streaked, several colonies (usually 4-6) were selected for identification and serotyping.

From each sampling from one part of the site, no more than about 4 m square,

Table 3. Serotypes of *E. carotovora* var. *carotovora* found associated with insects and potato stems in 1973.

Source[1]	Date of collection[2]	Serotype[3]
1. Bacteria isolated from insects[4]		
Leptocera sp. (11 bulked[5])	24. 7.73	3+4+5
Leptocera sp. (15 bulked)	24. 7.73	5
Fannia sp. (1 insect[6])	1. 8.73	3
Unidentified dipteran[7] (3 bulked)	5. 9.73	3
Fannia sp. (1 insect)	16.10.73	3
2. Bacteria isolated from potato stems[8]		
Potato stem[9] (Up-to-date)	10. 8.73	2
	10. 8.73	2
	10. 8.73	2
	10. 8.73	2
Potato petiole[10]	10. 8.73	3
Potato stem (Majestic)	15. 8.73	4
	15. 8.73	5

[1] *Quelle - Source;* [2] *Samlungsdatum - Date d'observation;* [3] *Serotyp - Sérotype;* [4] *Bakterien isoliert von Insekten - Bactéries isolées des insectes;* [5] *Insgesamt - Amas;* [6] *Insekt - Insecte;* [7] *Nicht identifizierte Dipteren - Diptères non identifiés;* [8] *Bakterienisolate von Kartoffelstengeln - Bactéries isolées des tiges de pomme de terre;* [9] *Kartoffelstengel - Tige de pomme de terre;* [10] *Kartoffelblattstiel - Pétiole de pomme de terre*

Tabelle 3. Serotypen von *E. carotovora* var. *carotovora*, die an Insekten und Kartoffelstengeln 1973 gefunden wurden.

Tableau 3. Sérotypes d'*E. carotovora* var. *carotovora* trouvés associés aux insectes et aux tiges de pomme de terre en 1973.

flies (*Leptocera* spp., *Fannia* spp. and unidentified dipterans) yielded *E. carotovora*. Out of 133 insects tested from this area, at least 5 must have been carrying the bacteria (it was impossible to tell the exact number because of bulking), whereas 205 insects from 11 other areas around Ingraston were not.

Soft rots well above ground level were discovered on 10 August on stems of 19 plants in a plot of cv. Up-to-Date. Another plant had a blackened petiole scar and samples were examined bacteriologically. On 15 August, in a plot of cv. Majestic, mechanically damaged stems with fluid exuding from them were sampled. *E. carotovora* was isolated from both lots of material; results of serotyping organisms from insects and plants are summarized in Table 3. These results showed that all 3 serotypes associated with the insects in July were subsequently found infecting potato plants in August, 17 and 22 days after insects had been caught at the waste dump and found contaminated. Although another serotype found on plants was not associated with the insects tested, the results pointed to the insects as a source of infection on the potato plants. Furthermore, insects found at the waste dump were still carrying soft rot coliforms in mid-October, long after the haulms had been destroyed with sodium chlorate in August and September, and the crop harvested. (As in 1972, 1973 was a dry harvesting season and haulms dried and bleached quickly.) It is known that insects of the genera *Leptocera* and *Fannia* are 'dirty' feeders, breeding in organic waste, and observations in the field revealed that these insects visited the crop, and were particularly attracted by fluid exuding from damaged stems.

On 16 October, the small part of the waste dump that yielded contaminated insects was dug over to a depth of about ½ m looking for any particular plant remains, such as potato tubers, which might have been a source of bacteria. All that was found was well decomposed vegetable matter except for a bulb of Dutch iris (*Iris hollandica*), which was infected with *E. carotovora* serotype 6, a serotype last found on potatoes during the 1971-72 storage period. The source of the bacteria associated with the insects from this site therefore remained undiscovered.

Storage period 1973-74

Only 3 stocks out of 340 contained soft rotted tubers. Two were infected with *E. carotovora*. Five isolates of *E. carotovora* (3 from 1 stock and 2 from another) were serotyped. All were serotype 1, a serotype found in the growing crop and on an insect in 1971, but not in the growing crop or on insects in 1973, although it was found on potato tubers from the plants growing in the dump in 1973.

During 1967-73, tuber infections were always found in stocks 2 or more years beyond the stem cutting stage of propagation, except in 1971 when tubers from 2 plants of first year stem cuttings were infected with *E. atroseptica*.

Growing season 1974

Insects were collected at the site of the rubbish dump at roughly fortnightly intervals, from 17 June to 9 October, and on 3 occasions in a nearby turnip crop. Over

Table 4. Serotypes of *E. carotovora* var. *carotovora* found associated with insects and potato stems in 1974.

Source[1]	Date of collection[2]	Serotype[3]
1. Bacteria isolated from insects[4]		
Leptocera sp. (4 bulked[5])	18. 7.74	3
Leptocera sp. (1 insect[6])	31. 7.74	2
Leptocera sp. (1 insect)	19. 8.74	8
Unidentified dipteran[7] (1 insect)	18. 9.74	6
2. Bacteria isolated from potato stems[8]		
Potato stems[9]	5. 9.74	2
	5. 9.74	2
	5. 9.74	2
	5. 9.74	2
	18. 9.74	6
	18. 9.74	8
	18. 9.74	8
	18. 9.74	6
	18. 9.74	9
	18. 9.74	2
	9.10.74	10
	9.10.74	10
	9.10.74	3
	9.10.74	6
	9.10.74	6

[1–9] *Siehe Tabelle 3 - Voir le tableau 3*

Tabelle 4. Serotypen von *E. carotovora* var. *carotovora*, die 1974 an Insekten und Kartoffelstengeln gefunden wurden.
Tableau 4. Sérotypes d'*E. carotovora* var. *carotovora* trouvés associés aux insectes et aux tiges de pomme de terre en 1974.

this period 639 insects (sometimes bulked according to genus, others as individuals) were tested bacteriologically. Four lots were found infected with *E. carotovora*; 3 from the same limited area of the dump as in 1973 and 1 from the turnip crop. No soft rot stem infections were seen in the growing potato crop or the turnips. Potato haulm was destroyed with a dinoseb spray during August and September, but some stems remained green, although they eventually rotted. After harvest, 64 of these stems (some of which included first year stem cuttings) were taken at random from the field on 3 occasions and tested bacteriologically; 15 yielded *E. carotovora*. Results of serotyping organisms from insects and stems are detailed in Table 4. These results show that all the serotypes found on the insects were also found on the potato stems left in the field after lifting, together with two additional serotypes (9 and 10) found only on potato stems. Serotype 2, found on potato haulm but not on insects in 1973, was found on an insect caught at the dump and on stems

Table 5. Number of plants grown, and number affected by blackleg in the period 1967-74.

Year[1]	Total number of plants grown[2]	Number affected by blackleg[3]
1967	1 000	0
1968	9 000	0
1969	120 000	1
1970	130 000	0
1971	170 000	15
1072	170 000	42
1973	150 000	12
1974	100 000	3

[1] *Jahr - Année;* [2] *Gesamtzahl der angebauten Pflanzen - Nombre total de plantes ayant végété;* [3] *Zahl der Pflanzen mit Schwarzbeinigkeit - Nombre de plantes affectées par la jambe noire*

Tabelle 5. Gesamtzahl der Pflanzen und Anzahl der Pflanzen mit Schwarzbeinigkeitssymptomen in den Jahren 1967-1974.
Tableau 5. Nombre de plantes ayant végété et nombre de plantes affectées par la jambe noire au cours de la période 1967-1974.

in 1974. It is concluded that despite the finding of serotypes 9 and 10 only on stems, the observations again strongly suggest that infections with *E. carotovora* in the crop originated from insects dispersing from the dump of waste nearby.

Infection of potato plants and stored tubers at Ingraston with Erwinia carotovora var. atroseptica

Over the years, a very small number of potato plants showing typical blackleg symptoms occurred in crops at Ingraston, mostly in 1972. Details are given in Table 5. All affected plants were tested bacteriologically and found infected with *E. atroseptica*. Infections were often associated with certain clones and except for 1 case were found in plants grown from tubers at least 2 years beyond the stem cutting stage. In 1972, when 42 blackleg infected plants were found, 26 were confined to 4 plots (each of a different cultivar) out of 367 plots. It is noteworthy that the 1972 infections followed the finding of 13 stocks carrying *E. atroseptica* infection in the 1971-72 storage period as discussed below.

Tubers in storage were also found infected with *E. atroseptica*; details of the number of stocks in store and the number of stocks found infected are given in Table 6. Compared with the total number of stocks grown, very few were infected with this organism and indeed notably fewer than with *E. carotovora*. In each stock only a few tubers (usually 2 or 3) were affected with soft rot or heel end necrosis.

The origin of the infections with *E. atroseptica* remains unknown; the organism was never found in association with insects caught in and around Ingraston. It might be argued that the bacteriological procedure used for testing the cuttings failed to detect latent infection. Whereas this is possible, the figures show that since 1967,

Table 6. Occurrence of *E. atroseptica* in stocks in storage over the period 1967-74.

Year[1]	Total number of stocks[2]	Number of stocks infected with *E. atroseptica*[3]
1967-70	1636	1
1971-72	501	13*
1972-73	367	0
1973-74	340	1

* 8 of the stocks were also infected with *E. carotovora* - *8 dieser Kartoffelpartien waren auch mit E. carotovora infiziert* - *8 de ces lots étaient aussi infectés par E. carotovora*

[1] *Jahr* - *Année*; [2] *Gesamtzahl der Kartoffelpartien* - *Nombre total de lots*; [3] *Anzahl der mit E. atroseptica infizierten Kartoffelpartien* - *Nombre de lots infectés par E. atroseptica*

Tabelle 6. Auftreten von *E. atroseptica* in Kartoffelpartien im Lager während der Jahre 1967-1974.
Tableau 6. Observations d'*E. atroseptica* sur des lots en stockage au cours de la période 1967-1974.

only 2 of some 20 000 tested cuttings have been found infected with soft rot coliforms; in both instances, *E. carotovora* was isolated.

Discussion

Production of stem cuttings and multiplication of progeny at Ingraston farm over 8 years has shown that it is possible to produce crops essentially free from infection with soft rot coliform bacteria on a commercial scale. Although reinfections have occurred, the numbers have been very small compared with the total production, and reached very much lower levels of infection that those of ordinary commercial stocks. It is also especially noteworthy that, except in 1972, the organism most frequently found was *E. carotovora* and not *E. atroseptica*. However infections which occurred on VTSC material have provided an opportunity to investigate how soft rot coliforms may survive and spread in the environment. Up till now this has been impossible because alternative ways of survival and transmission have been obscured by the universal infection of ordinary commercial tubers with soft rot coliform bacteria.

Observations that insects can transmit soft rot coliform bacteria are not new and scattered information can be found over the years throughout phytopathological and entomological literature. As long ago as 1926, Leach (1926) showed that the dipterous insect *Hylemyia cilicrura* could transmit *Erwinia* spp. to potatoes, work which suggested that the bacteria persisted throughout the life cycle of the insect. *Hylemyia cilicrura* occurs in Britain, but it was never encountered in this study. There are also reports of dipterans and other insects acting as possible vectors, for instance in relation to soft rot of crucifers and *Erioischia brassicae* (Johnson, 1930; Doane &

Chapman, 1964); and heart rot of celery associated with attacks by the leaf mining insect *Scaptomyza graminum* (Ogilvie et al., 1935). Chiu et al. (1958) reported that various insects, including bees, transmitted soft rot coliform bacteria in cabbage stores, and Tamimi & Banfield (1969) described transmissions of unspecified soft rot organisms from various plants (including potato) to lettuces by species of *Drosophila*. Molina et al. (1974), demonstrated that *Drosophila melanogaster*, contaminated with *E. atroseptica*, transmitted infection to damaged potato stems very efficiently. In a more recent experiment, (Harrison & Graham, unpublished) insects (including *Leptocera* spp. and *Drosophila* spp.) caught at a waste potato dump were placed in a cage containing 5 potato plants with damaged stems. Bacteriological tests showed that 4 out of 5 damaged plants became infected with *E. carotovora* after 3 days. This emphasizes the ease with which these insects can transmit bacteria to damaged parts of plants.

Although the number of insects caught carrying soft rot coliform bacteria at the dump was small compared with those not contaminated, the sample must represent only a fragment of the total population dispersing from the dump throughout the growing season. Nevertheless the observations are also in accord with an epidemiological picture involving relatively few infected insects, otherwise a much larger number of plants at Ingraston probably would have been found infected with *E. carotovora*. Serotyping the isolates of *E. carotovora* indicated that isolates from insects and plants were identical in many cases, from which it may be inferred that the plants were infected by organisms carried by the insects, though other means of transmission cannot be ruled out. It is also clear that there are many serotypes of soft rot coliforms and a larger number of antisera would be of help in further epidemiological research. In this connection it is noteworthy that serotyping did not always indicate a relationship between the bacteria found in the growing crop and those in diseased stored tubers, although not all isolates from tubers were serotyped to conserve the limited supplies of antisera. More studies on these lines are clearly required. Additionally, the stocks in which affected tubers were found during storage were different from the stocks in which infection was found in the growing crop, for all stocks found infected in the growing season are destroyed.

The precise nature of the association of *Erwinia* spp. with *Leptocera* spp., *Drosophila* spp., *Fannia* spp. and other insects is not known, but it may be that insects which live and breed in decaying organic matter, merely become contaminated with *Erwinia* spp. if these happen to be present in the organic waste. Insects including those of the above three genera have been collected from several other heaps of rotting vegetable matter and a manure heap, but soft rot coliforms have not been isolated from them.

It is obvious that if insects are contaminated with *Erwinia* spp., they could act as vectors, for they disperse from their breeding grounds and presumably may travel considerable distances. Yerrington & Warner (1961) showed that *Drosophila melanogaster* could move about 8 km in 24 h; Lempke (1962) found *Fannia canicularis* could travel up to 70 km; and Yates & Lindquist (1952) demonstrated that *Musca*

domestica could move as far as 32 km. Their efficiency as vectors will depend on many other uncertain factors; for instance, the period of survival of soft rot coliforms in association with insects is not known, but tests show it must be at least 24 h.

In summation, the data provide compelling but not conclusive evidence that infections with *E. carotovora* found in VTSC potato plants at Ingraston were established by insects associated with a dump of vegetable matter nearby. Once infection becomes established on plants the population of bacteria could build up on them, especially under favourable conditions (particularly wet weather) and spread could then take place within the crop. Internal spread could occur in several ways, such as by further insect transmission, by contaminated machinery and by rain splash. Establishment of primary foci by insects from outside the crop and probably also transmission within the crop (as insects are unlikely to live in the crop) could not be prevented by spraying with insecticides. The only way to try to ensure freedom from infection is by good hygienic measures, including the disposal of vegetable waste (especially waste potato tubers) by methods other than dumping.

A noteworthy observation is that rotting, but still green, stems found in the field late in the season (and even after the crop had been lifted) in 1971 and 1974 often yielded soft rot coliforms, especially *E. carotovora*. This indicates substantial build-up of stem infection can occur, particularly in wet seasons, and emphasises the need to use an efficient chemical haulm killer. Furthermore, every effort should be made to ensure complete coverage of the crop by the haulm killer as part of general hygienic measures.

This study has not thrown any fresh light on the source of *E. atroseptica*. Perhaps some simple hygienic procedure has been overlooked or contaminated flies reached the crop from far away, but equally there may be as yet undiscovered (or unconfirmed) ways in which soft rot coliform bacteria can spread in the environment. It has, however, highlighted the complexity of the epidemiology of diseases caused by soft rot coliforms and in particular the association of causal organisms with insects.

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Zusammenfassung

Erneutes Auftreten von Infektionen mit coliformen Nassfäuleerregern an Kartoffelstengelstecklingen: Eine epidemiologische Studie in der zentralen Saatgutvermehrung in Schottland in den Jahren 1967-74

1967 wurde 30 km von Edinburg entfernt unter des Aussicht des Department of Agriculture and Fisheries for Scotland (DAFS) mit der Produktion von Saatkartoffeln, die von ge-

sundem Nachbau von Stengelstecklingen stammten begonnen. Die Produktion wurde seitdem von spezialisierten Vermehrern als VTSC-Saatgut gesteigert. (VTSC = Nachkommen von virusgetesteten Stengelstecklingen): VTSC ist der höchste Grad im DAFS Anerkennungsschema. Das Ziel dieser Produktionsart ist die Ausschaltung von Krankheitserregern, die im wesentlichen von der Knolle übertragen werden; in diese Gruppe gehören die coliformen Nassfäulebakterien.

Seit 1967 wurde in dem Ausgangssaatgut der DAFS sehr genau das Auftreten von Infektionen mit *Erwinia carotovora* var. *carotovora* (*E. carotovora*) und *Erwinia carotovora* var. *atroseptica* (*E. atroseptica*) überwacht. Während der Jahre 1967-1974 wurde eine kleine Zahl von Stengeln und Knollen infiziert mit diesen Erregern gefunden, aber mit Ausnahme von 1972, war der vorherrschend auftretende Erreger *E. carotovora*, während im gewöhnlichen Konsumanbau in Schottland *E. atroseptica* zu finden war. Infektionen mit *E. carotovora* sind sehr deutlich von typischen Schwarzbeinigkeitssymptomen zu unterscheiden; gewöhnlich findet man grünliche nassfaule Stellen an beschädigten Blättern oder gegen Ende der Wachstumsperiode faulende Stengel.

Im August und September 1971 wurde eine kleine Zahl von Pflanzen mit einem Feld von 4000 Stengelstecklingen hauptsächlich mit *E. carotovora* infiziert gefunden (obwohl auch *E. atroseptica* in geringem Ausmass vorhanden war) und Insekten (einschliesslich *Drosophila* spp. und *Vespa* spp.), die zwischen den Pflanzen gefangen wurden, waren mit *E. carotovora* behaftet. Es konnte nicht festgestellt werden, ob die Pflanzen durch die Insekten infiziert wurden oder umgekehrt, aber um zu prüfen, ob irgendein Zusammenhang zwischen den Organismen der Insekten und der Pflanzen bestand, wurde der Serotyp der Isolate von beiden Herkünften bestimmt. In Tabelle 1 sind die Erreger, die zur Herstellung der Antiseren verwendet wurden aufgeführt und Tabelle 2 zeigt die in den Jahren 1971-1974 gefundenen Serotypen. Es ergab sich, dass die Serotypen, die von den Insekten isoliert wurden, mit den meisten der Isolate der Pflanzen identisch waren. 1972 wurden keine mit *E. carotovora* infizierten Kartoffelstengel gefunden, aber ein nahe gelegener Rübenbestand war infiziert und Insekten, die in diesem Bestand gefangen wurden, enthielten den selben Erreger. Auf dem Feld wurde eine Saugfalle aufgestellt, sie war aber ungeeignet, da die Insekten in ihr schnell starben und austrockneten und nur weinig Bakterien isoliert werden konnten. 1973 wurden auf einem nahegelegenen Gemüseabfallhaufen Dipteren, einschliesslich *Leptocera* spp. und *Fannia* spp., gefangen, die bereits einige Wochen bevor Infektionen mit diesem Erreger an Kartoffelstengeln auftraten, mit *E. carotovora* kontaminiert waren (Fig. 1). Tabelle 3 zeigt die mit dem Erreger kontaminierten Insekten und Pflanzen und die Serotypen. Im wesentlichen ähnlich Ergebnisse wurden 1974 erhalten, obwohl Stegelinfektionen nur an alten Stengeln nach der Ernte beobachtet wurden; die Ergebnisse sind in Tabelle 4 zusammengestellt. Diese Ergebnisse liefern den zwingenden aber nicht endgültigen Beweis, dass eine Infektionsquelle mit *E. carotovora* kontaminierte Insekten von Abfallhaufen sind, die sich auf beschädigten Stengeln, Blättern oder alten Kartoffelstengeln verteilen und sich gleichzeitig davon ernähren.

Während der Jahre 1967-1974, vor allem 1972, wurden einige Kartoffelpflanzen infiziert mit *E. atroseptica* gefunden (Tabelle 5). Diese Infektionen traten im allgemeinen nur an Pflanzen, 2 oder mehr Jahre nach der Bildung der Stengelstecklinge, auf und sie schienen mit bestimmten Klonen verbunden zu sein. So wurden auch nur wenige Partien von Kartoffelknollen, die mit diesem Erreger infiziert waren, im Lager gefunden (Tabelle 6). Der Ursprung von *E. atroseptica* ist unbekannt, es wurden niemals kontaminierte Insekten in Ingraston festgestellt.

Die Ergebnisse erhellen die Bedeutung einfacher Hygienevorschriften, um Reinfektionen mit coliformen Nassfäuleerregern zu verhindern, vor allem durch Vermeidung von Ansammlungen faulender Gemüseabfälle (vor allem Kartoffeln) auf Farmen. Ebenso sollte ein wirksames chemisches Krautabtötungsmittel verwendet werden, um einen möglichen Ausbruch von Stengelinfektionen in der späten Wachstumsperiode zu verhindern. Die Epidemiologie der Nassfäulen verursacht durch *Erwinia* spp. ist noch nicht völlig geklärt und es können noch andere Möglichkeiten für die Verbreitung dieser Erreger bestehen.

Résumé

Réapparition des infections bactériennes de pourriture molle dans les boutures de tiges de pomme de terre: une étude épidémiologique à la Station de production des plants de base en Ecosse pendant les années 1967-74

La production de plants de base, indemnes de maladie, obtenus à partir de boutures de tige, a été commencée en 1967 par le Ministère de l'Agriculture écossais (DAFS), dans une station située à 30 km d'Edimbourg. La descendance a, depuis, été multipliée par des agriculteurs producteurs spécialisés de semences VTSC (boutures de tige testées pour verifier l'absence de virus), la classe VTSC étant la plus élevée dans le plan de certification des semences de pomme de terre du DAFS. Cette pratique a pour but d'éliminer tous les organismes responsables de maladies, qui sont essentiellement transmis par les plants; les bactéries coliformes de la pourriture molle appartiennent à ce groupe.

Depuis 1967, les plants de base du DAFS ont été soigneusement protégés contre les infections d'*Erwinia carotovora* var. *carotovora* (*E. carotovora*) et d'*Erwinia carotovora* var. *atroseptica* (*E. atroseptica*). Au cours de la période 1967-1974, un petit nombre de tiges et de tubercules ont été trouvés infectés par ces organismes, mais exception faite pour l'année 1972, *E. carotovora* était le plus communément présent, alors que dans les lots commerciaux, en Ecosse, c'est *E. atroseptica* qui prédomine. Les infections par *E. cartovora* sont tout à fait distinctes de la jambe noire typique - pourritures molles habituellement verdâtres sur les feuilles au niveau de blessures ou pourritures de tiges sénescentes en fin de période de croissance.

En août et septembre 1971, sur 4000 boutures de première année plantées dans le champ, quelques unes étaient infectées, principalement par *E. carotovora* (*E. atroseptica* n'était présent que sur une petite surface) et les insectes capturés sur les plantes (y compris *Drosophila* spp. et *Vespa* spp.) étaient contaminés par *E. carotovora*. Il était impossible de dire si les plantes avaient été infectées par les insectes ou vice-versa; mais, pour voir s'il y avait une relation, des isolations de chacune des deux sources furent sérotypées.

Les organismes utilisés pour produire l'antisérum sont donnés dans le tableau 1 et les sérotypes trouvés pendant la période 1971-1974 sont détaillés dans le tableau 2. Il a été montré que les sérotypes isolés à partir des insectes étaient identiques à la plupart de ceux isolés à partir des plantes. En 1972, aucune tige de pomme de terre n'était infectée par *E. carotovora*, mais, dans les environs, une récolte de navet était infectée et les insectes vivants capturés dans la parcelle portaient le même organisme. Un piège à insectes a été installé dans le champ, mais ce nu fut pas un succès car les insectes, qui mouraient et se desséchaient trop rapidement, produisaient peu de bactéries. En 1973, des insectes diptères, y compris *Leptocera* spp. et *Fannia* spp., capturés tout près d'un dépôt de détritus de matière végétale, étaient contaminés par *E. carotovora* plusieurs semaines avant que les infections par cet organisme se manifestent sur les tiges de pomme de terre. Les insectes et les plantes portant cet organisme et les sérotypes sont mentionnés dans le tableau 3. Globalement, des résultats similaires ont été obtenus en 1974, bien que les symptômes sur tiges n'apparurent que sur de vieilles tiges après l'arrachage de la récolte; les résultats sont donnés dans le tableau 4.

Sans être une preuve définitive, ces données montrent qu'une source des infections par *E. carotovora* pourraient être des insectes contaminés, associés aux tas de déchets, les insectes disséminant l'organisme après l'avoir prélevé sur les feuilles au niveau des blessures ou sur les tiges sénescentes.

Au cours de la période 1967-1974, quelques plantes de pomme de terre ont été trouvées infectées par *E. atroseptica*, spécialement en 1972, comme le montre le tableau 5. Ces infections n'ont généralement été observées que sur des plantes de deux ou plusieurs années au-delà du stade bouturage et elles avaient tendance à être associées à certains clones. Comparativement, très peu de lots de tubercules de pomme de terre, en magasin, ont été trouvés infec-

tés par cet organisme (tableau 6). L'origine d'*E. atroseptica* reste inconnue; à Ingraston, il n'a jamais été trouvé associé aux insectes.

Ces résultats mettent clairement en évidence l'importance de simples précautions pour éviter la recontamination par les bactéries coliformes de la pourriture molle, spécialement en évitant les accumulations de déchets de matière végétale (en particulier de pommes de terre), dans les fermes. Un défanant efficace pourrait aussi être appliqué pour prévenir de possibles infections de tiges, tard dans la saison. Toutefois, il est aussi mentionné que l'épidémologie des pourritures molles causées par *Erwinia* spp. n'est pas complètement connue, et qu'il peut y avoir d'autres moyens de dissémination de ces organismes dans le milieu ambiant.

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