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The effect of *Corynebacterium sepedonicum* on symptoms and yield of four potato cultivars

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Summary

Inoculating the roots of potato cvs Ostara, Laila, Kerrs Pink, and Pimpernel with *Corynebacterium sepedonicum* caused severe rotting of tubers and between 12 and 51 % decrease in yield. There was a significant cultivar/infection interaction. The numbers of tubers decreased significantly except for cv. Laila, which also showed little rotting of tubers, and the average tuber weight decreased most in cv. Pimpernel. The concentration of *C. sepedonicum* was high in most inoculated tubers including those not showing symptoms.

Introduction

Potato ring rot, caused by *Corynebacterium sepedonicum* (Spiek. et Kotth.) Skapt. et Burkh. is a threat to potato production in many countries. Infected mother tubers are the most important source of the bacteria and means of their dispersal. In Norway a major effort is being made to eliminate the disease by producing pathogen-free seed potatoes from meristem tip cultures. Strict measures are being taken to avoid contamination of these new lines by *C. sepedonicum* from old lines of seed potatoes that may have low levels of infection. Other potential measures that might help to control the disease include the use of resistant genotypes. However, little attention has been paid to their use since the works of Bonde et al. (1942, 1947, 1949) that resulted in the development of cultivars that were resistant, had desirable marketing qualities and were grown for more than seven years with no apparent loss in resistance. Unfortunately, it was found that resistant cultivars could be symptomless carriers of the bacteria and thus a constant threat to susceptible cultivars. This paper presents the results of an investigation of the effect of ring rot infection on symptom development and yield on four potato cultivars commonly grown in Norway.

Materials and methods

At the beginning of May, seed tubers of cvs Ostara, Laila, Kerrs Pink, and Pimpernel, (first and second early, main and late main crops respectively), known to be free from the ring rot pathogen, were sprouted in trays filled with moist perlite and kept in the glasshouse at 20 °C. After four weeks, when the shoots were 5–10 cm and the roots

10–20 cm long, the perlite was washed away in running water and the roots severed with scissors ca 1 cm from their tips. The cut roots were dipped either in sterile distilled water as a control treatment, or for 60 seconds in a suspension of ca 10^{10} cells/ml of *C. sepedonicum*. The inoculum was prepared by homogenizing tubers that had typical ring rot symptoms in macerating fluid (Lelliott et al., 1976) diluted 1:1 in distilled water. Immediately after inoculation, the plants were hand-planted in the field.

The design of the field experiment was based on a latin square of cultivar plots, each split to give sub-plots planted with uninoculated (control) or inoculated plants 30 cm apart in 60-cm-spaced rows. The sub-plots each consisted of one row of 10 plants and they were separated by an unplanted row to give 60-cm boundaries. The trial was harvested in August and September. All tubers were weighed and examined for external and internal symptoms. The presence of *C. sepedonicum* in tubers was confirmed by the gram-reaction (Dowson, 1957) and by indirect fluorescent antibody staining (IFAS), carried out as follows. Antiserum produced from live *C. sepedonicum* cells was fractioned (Cherry, 1970). Drops of air-dried potato juice on slides, counterstained with gelatine-rhodamine conjugate (Bohlool & Schmidt, 1968), were stained with the immunoglobulin by using the indirect technique (Slack et al., 1978). Stained slides were examined with a Zeiss microscope at 800 × magnification. From each slide, ten microscope fields were randomly chosen, and the concentration of *C. sepedonicum* determined to be nil, <100 or >100 cells per microscope field. From inoculated plots every tuber was tested, from control plots 30 tubers were chosen at random and tested for freedom from infection according to Lelliott et al. (1976), except that IFAS stain was used instead of the eggplant test. From each batch five slides were examined.

Results

Root-dip inoculation resulted in ring rot infection in all plants and *C. sepedonicum* could be detected in all tubers derived from them, regardless of symptom development. In contrast, the bacteria were never detected in tubers from control plants.

Inoculation substantially and significantly decreased yield, cv. Pimpernel being the most seriously affected (Table 1), and there was significant cultivar/infection interaction. Except for cv. Laila, inoculation also significantly decreased tuber numbers (Table 2), especially in cv. Kerrs Pink, whereas cv. Pimpernel showed the greatest reduction in average tuber weight (Table 3).

High tuber contamination levels were associated with severe ring rot of completely or partly rotted tubers that could easily be detected when they were lifted (Table 4). When tubers without external symptoms were cut with a knife shortly after lifting, ring rot symptoms of varying severity could be seen in the vascular bundles. External tuber symptoms were rare in cv. Laila and, although it had the highest percentage with internal symptoms, it nevertheless had nearly twice as many wholly symptomless tubers as the other three cultivars which in turn had similar levels of internal and external symptom development.

The concentrations of *C. sepedonicum* were always high in tubers of inoculated plants, regardless of symptom development, and they were consistently very high in tubers with symptoms, either external or internal, but there were no significant differences between cultivars.

Table 1. The effect of inoculation with *C. sepedonicum* on tuber yield (t/ha) from four potato cultivars.*

	Ostara	Laila	Kerrs Pink	Pimpernel
Inoculated[1]	21.9	31.4	20.9	23.7
Control[2]	33.8	35.9	39.9	48.9
Reduction in yield (%)[3]	35.2	12.5	47.6	51.5

* Differences between inoculated and control significant at $P < 0.001$, and cultivar/infection interaction significant at $P < 0.025$ - *Unterschiede zwischen inokuliert und Kontrolle bei* $P < 0{,}001$ *und bei Sorte/Infektion-Wechselwirkung bei* $P < 0{,}025$ *signifikant - Différences significatives entre plante inoculée et témoin à* $P < 0{,}001$ *et interaction significative variété/infection à* $P < 0{,}025$.

[1] *Inokuliert - Inoculée;* [2] *Kontrolle - Témoin;* [3] *% Ertragsreduktion - % de réduction du rendement*

Tabelle 1. Einfluss einer Inokulation mit *C. sepedonicum* bei vier Kartoffelsorten auf den Knollenertrag (t/ha).
Tableau 1. L'effet sur le rendement en tubercule (t/ha) pour 4 variétés de pommes de terre inoculées avec *C. sepedonicum.*

Table 2. The effect of inoculation with *C. sepedonicum* on average number of tubers/plant of four potato cultivars.*

	Ostara	Laila	Kerrs Pink	Pimpernel
Inoculated[1]	6.7	12.9	9.8	13.0
Control[2]	10.0	12.7	17.8	15.2

* Except for cv. Laila, differences between inoculated and control significant at $P < 0.001$ and cultivar/infection interaction significant at $P < 0.005$ - *Mit Ausnahme der Sorte Laila waren die Unterschiede zwischen inokuliert und Kontrolle bei* $P < 0{,}001$ *und bei Sorte/Infektion bei* $P < 0{,}005$ *signifikant - A l'exception de la variété Laila, les différences entre plante inoculée et témoin sont significatives à* $P < 0{,}001$ *et l'interaction variété/infection significative à* $P < 0{,}005$.

[1,2] *Siehe Tabelle 1 - Voir tableau 1*

Tabelle 2. Einfluss einer Inokulation mit *C. sepedonicum* auf die durchschnittliche Zahl Knollen/Pflanze bei vier Kartoffelsorten.
Tableau 2. L'effet sur le nombre moyen de tubercules par plante pour 4 variétés de pommes de terre inoculées avec *C. sepedonicum.*

Table 3. The effect of inoculation with *C. sepedonicum* on average tuber weight of four potato cultivars.

	Ostara	Laila	Kerrs Pink	Pimpernel
Inoculated[1]	59	44	38	33
Control[2]	61	51	41	58

[1,2] *Siehe Tabelle 1 - Voir tableau 1*

Tabelle 3. Einfluss einer Inokulation mit *C. sepedonicum* auf das durchschnittliche Knollengewicht von vier Kartoffelsorten.
Tableau 3. L'effet sur le poids moyen de tubercules pour 4 variétés de pommes de terre inoculées avec *C. sepedonicum.*

Table 4. Symptom development in four potato cultivars inoculated with *C. sepedonicum*, and concentrations of *C. sepedonicum* in symptomless tubers from inoculated plants (% tubers/cultivar).

	Ostara	Laila	Kerrs Pink	Pimpernel
Tubers with external and internal symptoms[1]	31.3	0.7	36.7	29.2
Tubers with only internal symptoms[2]	44.8	57.4	39.8	43.1
Symptomless tubers[3]	23.9	41.9	23.5	27.7
Symptomless tubers with >100 cells/ microscope field[4]	83.3	75.5	61.9	77.9
Symptomless tubers with <100 cells/ microscope field[5]	16.7	24.5	38.1	22.1

[1] *Knollen mit externen und internen Symptomen – Tubercules avec des symptômes externes et internes;* [2] *Knollen ausschliesslich mit internen Symptomen – Tubercules avec seulement des symptômes internes;* [3] *Symptomlose Knollen – Tubercules sans symptômes;* [4] *Symptomlose Knollen mit >100 Zellen pro Mikroskopierfeld – Tubercules sans symptômes avec plus de 100 cellules par champ microscopique;* [5] *Symptomlose Knollen mit <100 Zellen pro Mikroskopierfeld – Tubercules sans symptômes avec moins de 100 cellules par champ microscopique*

Tabelle 4. Symptomenentwicklung in vier mit *C. sepedonicum* inokulierten Kartoffelsorten sowie Konzentrationen von *C. sepedonicum* in symptomlosen Knollen von inokulierten Pflanzen (% Knollen/Sorte).

Tableau 4. Développement des symptômes pour 4 variétés de pommes de terre inoculées avec *C. sepedonicum* et concentration de *C. sepedonicum* dans les tubercules sans symptôme et provenant de plantes inoculées (% tubercules/variété).

A few plants, in particular cv. Ostara, developed wilt symptoms but these were usually diffuse and atypical making it difficult to distinguish them from foliage symptoms caused by other agents.

Discussion

Infection of potato plants by *C. sepedonicum* reduced yield in these experiments by up to 51 %. Similar losses in commerce would be more serious; only 24 to 42 % of tubers from infected plants were free from symptoms, so that saleable yield losses would have been in the range 60–75 %. Yield losses of this order (40–70 %) were reported by Nelson & Torfason (1974) and Nelson & Howard (1982) when cvs Netted Gem and Russet Burbank were knife-inoculated with *C. sepedonicum*. Fortunately, such heavy losses are unusual in practice, and in Norway losses due to tuber rotting are of minor importance. However, a crop entered for certification as seed potatoes will be rejected and downgraded to ware if one tuber with ring rot symptoms is found among 400 tested, with corresponding economic loss to the grower.

The reduction of yield in cv. Laila was small and it was caused by a reduction in weight of tubers, whereas in the other cultivars the more substantial yield losses were caused by a decrease in the numbers of tubers. This may indicate that the development of infection in cv. Laila started after tuber initiation, whereas in the other cultivars it may have started earlier and thereby influenced tuber initiation. The small numbers of tubers showing external symptoms due to extensive rotting in cv. Laila would also support this hypothesis. Although between 24 and 42 % of tubers from infected plants were free from symptoms, the concentration of *C. sepedonicum* was high (>100 cells/ microscope field) in the majority of these tubers. Attempts in preliminary experiments to estimate by isolation the bacterial concentration per g of tuber tissue were unsuccessful because the slow growth of *C. sepedonicum* made plating inefficient. Nelson (1982) examined the lower stems of latent infected potato plants and estimated that there were more than 10^9 cfu of the ring rot pathogen per g of stem tissue, a figure that may also apply to latent infected tubers.

Of the four cultivars tested, cv. Laila might be suitable for breeding for resistance to the ring rot pathogen because it had the smallest reduction in yield compared with the other cultivars. However, high level of internal tuber symptoms and high concentrations of *C. sepedonicum* in the majority of symptomless tubers from inoculated plants argue against its use in breeding.

It may also be argued that cultivars resistant to the ring rot pathogen are of little value as long as they are symptomless carriers of the bacteria and thus a constant threat to susceptible cultivars. On the other hand, once the pathogen has been established in an area, it may be an impossible task to eradicate it, even when strict control measures are taken. Under such circumstances extensive use of resistant cultivars, even with low levels of infection, may be a useful way to reduce the damage caused by the pathogen. As reported by a Committee of the Potato Association of America (Anon., 1957) and Nelson (1982), low levels of infection may persist for several generations. Long-term field experiments, to be reported later, indicate that under conditions in Norway, low levels of infection (<10 cells/ microscope field) may persist for many generations, with apparently no detectable effect on yield or any symptom development.

The seed tubers used in the present study were free from ring rot; the inability to detect *C. sepedonicum* in progeny tubers from control plots, despite their proximity to plots of infected plants, suggests that spread of the bacteria in the field may not be common.

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Zusammenfassung

Einfluss von Corynebacterium sepedonicum auf Symptome und Ertrag bei vier Kartoffelsorten

Wurzeln von gekeimten Saatknollen der Sorten Ostara, Laila, Kerrs Pink und Pimpernel wurden durch Tauchen in Suspensionen von *Corynebacterium sepedonicum* inokuliert. Dies

erbrachte einen beträchtlichen und signifikanten Ertragsrückgang, vor allem bei der Sorte Pimpernel (Tabelle 1); desweiteren zeigte sich eine signifikante Sorte/Infektion-Wechselwirkung. Inokulation ergab, mit Ausnahme von Laila, eine signifikante Senkung der durchschnittlichen Knollenzahl (Tabelle 2); am grössten war der Rückgang bei Kerrs Pink, während das durchschnittliche Knollengewicht am meisten zurückging bei Pimpernel (Tabelle 3). Der Ertragsrückgang bei Laila erfolgte durch Reduktion des durchschnittlichen Knollengewichtes, bei den anderen Sorten jedoch durch Rückgang der durchschnittlichen Knollenzahl. Diese Beobachtung lässt darauf schliessen dass die Entwicklung der Infektion bei Laila nach der Knollen-Initiierung einsetzte, während sie bei den anderen Sorten schon früher einsetzte und somit die Knollenzahl beeinflusste.

Im Gegensatz zu den anderen Sorten zeigten einige Tochterknollen von Laila äussere Symptome, in Verbindung mit starker Fäule, während in der Entwicklung interner Symptome nur geringe Unterschiede zwischen den Sorten gefunden wurden (Tabelle 4). Dennoch waren die Konzentrationen von *C. sepedonicum* in den meisten symptomlosen Tochterknollen inokulierter Pflanzen hoch (Tabelle 4).

Die Ergebnisse zeigen dass die Sorte Laila gegen eine *C. sepedonicum*-Infektion resistenter als die anderen geprüften Sorten ist, dass jedoch hohe Konzentrationen des Erregers in symptomlosen Knollen von inokulierten Pflanzen gegen eine Verwendung bei der Züchtung sprechen.

Résumé

L'effet de Corynebacterium sepedonicum et le rendement de quatre variétés de pommes de terre

Des racines de tubercules de semence germés (variétés Ostara, Laila, Kerrs Pink et Pimpernel) sont inoculées par immersion dans des suspensions de *Corynebacterium sepedonicum*. Cela provoque une diminution conséquente et significative du rendement, en particulier pour la variété Pimpernel (tableau 1), et il existe une interaction significative variété/infection. L'inoculation diminue significativement le nombre moyen de tubercules (tableau 2) sauf pour la variété Laila; la diminution est la plus importante pour la variété Kerrs Pink, tandis que le poids moyen de tubercules diminue le plus pour la variété Pimpernel (tableau 3). La diminution du rendement pour la variété Laila est provoquée par une réduction du poids de tubercules. Cette observation suggère que le développement de l'infection pour la variété Laila débute après l'initiation de tubérisation, alors que pour les autres variétés, elle commence plus tôt et de ce fait influence le nombre de tubercules.

Peu de tubercules fils de la variété Laila montrent des symptômes externes associés à une pourriture importante en comparaison avec les autres variétés, alors que de petites différences s'observent entre toutes les variétés au niveau du développement de symptômes internes (tableau 4). Les concentrations de *C. sepedonicum* sont cependant élevées dans la plupart des tubercules fils ne présentant pas de symptômes et provenant de plantes inoculées (tableau 4).

Les résultats montrent que la variété Laila est plus résistante à l'infection de *C. sepedonicum* que les autres variétés testées, mais les fortes concentrations du pathogène dans les tubercules sans symptôme provenant de plantes inoculées empêchent son utilisation en multiplication.

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