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Epidemiology of *Clavibacter michiganensis* subsp. *sepedonicus* in potato under European conditions: population development and yield reduction

Die Epidemiologie von *Clavibacter michiganensis* subsp. *sepedonicus* in Kartoffel unter europäischen Verhältnissen: Entwicklung der Population und Ertragsverlust

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Summary

The population development of *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) using fluidal strain (NCPPB 4053) and non-mucoid strain (NCPPB 3898) were monitored by IF cell staining method in potato cultivars 'Hansa' and 'Desiree' under field conditions in Denmark and Finland. The influence of Cms on potato yield in 'Hansa' was also examined under field conditions in Finland. Population development was generally similar in potato fields in Denmark and Finland, but a higher bacterial density and a higher number of symptomatic plants were found in Finland than in Denmark. Inoculation of potato tubers with the fluidal Cms strain NCPPB 4053 resulted in higher densities of Cms in plants during the growing season than inoculation with the non-mucoid strain NCPPB 3898. Population densities of the fluidal Cms strain (4053) increased rapidly in the beginning of the seasons, and reached its maximum ca. 80–100 days from planting in susceptible 'Hansa'. Lower levels of Cms were found in tubers of the more resistant 'Desiree' than in those of the susceptible 'Hansa'. Tuber and stem symptoms emerged 80–100 days after planting in 'Hansa', but no visual symptoms were observed in 'Desiree'. Severe infection reduced the tuber yield of 'Hansa' by 41–56 % in Finland.

Key words: Ring rot; field experiment; immunofluorescence cell-staining; antibiotic resistant mutants

Zusammenfassung

Die Entwicklung der Populationen von *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) (ein fließender Stamm – NCPPB 4053 und ein nicht schleimiger Stamm – NCPPB 3898) wurde unter Freilandbedingungen in Dänemark und Finnland mit der IF-Zellfärbemethode an den Kartoffelsorten 'Hansa' und 'Desiree' untersucht. Ferner erfolgte in Finnland eine Überprüfung des Einflusses von Cms auf den Knollenertrag von 'Hansa' im Freiland. Im Allgemeinen entwickelten sich die Populationen in den Kartoffelfeldern in Dänemark und Finnland ähnlich, aber in Finnland konnte eine stärkere Bakteriendichte und eine größere Zahl von symptomtragenden Pflanzen als in Dänemark beobachtet werden. Die Inokulation von Kartoffelknollen mit dem fließenden Cms-Stamm NCPPB

4053 hatte eine höhere Cms-Dichte in den Pflanzen während der Wachstumsphase zur Folge als die Inokulation mit dem nicht schleimigen Stamm NCPPB 3898. Die Populationsdichte des fließenden Cms-Stamms (4053) nahm zu Beginn der Vegetationsperiode rasch zu und erreichte bei der anfälligen Sorte 'Hansa' das Maximum 80–100 Tage nach dem Pflanzen. In den Knollen der resistenten Sorte 'Desiree' wurden weniger Bakterien gefunden als in denen der Sorte 'Hansa'. Symptome an den Knollen und sprossen tauchten an der Sorte 'Hansa' 80–100 Tage nach dem Pflanzen auf, aber an 'Desiree' erschienen keine sichtbaren Symptome. Schwere Infektionen reduzierten in Finnland den Knollenertrag bei 'Hansa' um 41–56 %.

Stichwörter: Bakterien-Ringfäule; *Clavibacter michiganensis* subsp. *sepedonicus;* Feldversuche; Immunofluoreszenz-Zellfärbung; Antibiotika-resistente Mutanten

1 Introduction

Bacterial ring rot of potato (*Solanum tuberosum* L.), caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), is considered a serious problem to the North American and North European seed potato production. It has also recently been found in southern parts of Europe, which suggests it is not restricted only to low temperature zones. In many cultivars, foliar symptoms appear as interveinal chlorosis followed by wilting and necrosis, while symptoms in tubers include breakdown of vascular tissues (De Boer and McCann 1990; Westra and Slack 1994). Several factors such as temperature, moisture, and soil fertility affect symptom expression (Logsdon 1967; Kawchuk et al. 1998). Furthermore, genotype and inoculum concentration have profound influence on both foliar and tuber symptom expression (Starr 1947; Bishop and Slack 1987; Westra and Slack 1994). However, the effect of dose as well as of cultivar seems to vary in different environments, and often in unpredictable ways (Westra and Slack 1994).

Bacterial ring rot is mainly controlled by zero tolerance policy through post-harvest inspections. The occurrence of latent Cms infections and the persistence of inadvertent inoculum sources have been considered as some reasons for our inability to eradicate ring rot based on visual symptom expression (Franc 1999). Recent new outbreaks both in southern and northern Europe suggest that much more information is needed on the epidemiology of this pathogen for effective eradication. Relatively little information is available on the population development of Cms under different climatic conditions in relation to symptom expression. Although Cms is a potential threat to European seed potato business, very few reports have been published on the epidemiological development of Cms or on the yield losses it is causing under different European conditions. In a joint European project, a multiple approach was initiated to reveal new information on the epidemiology of Cms under European conditions, which aims to provide new tools for effective management of the disease. The present study was designed to provide more information about the population development of Cms by comparing the bacterial development in two fields, in Denmark and Finland, and about the ability of Cms to cause yield losses under northern European conditions.

2 Materials and methods

2.1 Population development trial

Field experiments on population development of Cms were performed at two different locations, at the agricultural research station of Jokioinen (Agrifood Research Finland) in South-West-Finland and at the Research Centre Flakkebjerg near Slagelse in Denmark in 2000 and 2001. Two potato cultivars, 'Hansa' (susceptible to Cms) and 'Desiree' (moderately tolerant to Cms) were used in the experiments. Cms-free seed tubers were presprouted to a length of 0.5–1.0 cm before inoculation and planting. The tubers were sprout inoculated with the streptomycin-resistant Cms strain NCPPB 4053 (fluidal strain) and with the rifampicin-resistant Cms strain NCPPB 3898 (non-mucoid strain) by a syringe with a hypodermic needle. The spontaneous antibiotic resistant mutant strains were originally selected for isolation of Cms from soil or other microbe-rich substrates (Toivonen et al. unpublished data) and

used in this experiment because they were readily available. Cms was grown at room temperature on NCP-88 plates (De la Cruz et al. 1992) containing 25 µg ml^{-1} rifampicin or 50 µg ml^{-1} streptomycin (9-cm Petri dishes) for 3 days and suspended in 5 ml of 0.01 mol/l phosphate buffer at pH 7.4, resulting in a thick creamy suspension. Heavy bacterial concentration ≥ 10^9 cells ml^{-1} was used to ensure maximal number of infected daughter tubers in both potato cultivars. About 10–20 µl of suspensions were injected under every sprout of each tuber to a depth of 0.5–1.0 cm. Control tubers were similarly treated with sterile buffer. The inoculated tubers were kept overnight at room temperature before planting.

In Finland, a hilled row was established for each cultivar × strain combination in the ploughed field, and tubers were planted by hand with 25–30 cm spacing. Tubers were planted on 24 May in 2000 and on 1 June in 2001. Standard NPK (13 : 4 : 15) fertilizer 620 kg ha^{-1} (Kemira GrowHow, Finland), rimsulfuron (Titus, 250 g kg^{-1}, DuPont) and metribuzin (Sen2cor, 705 g kg^{-1}, Bayer) herbicide and fluazinam 0,4 l ha^{-1} (Shirlan, 500 g l^{-1}, Syngenta) fungicide treatments were applied during the growing seasons. The plots were not irrigated. For the monitoring of Cms development, five plants per treatment were sampled 4 times during the growing season at about 3 week intervals. At the fourth sampling time in 2001, 10 plants were harvested from every treatment. Symptoms were monitored during sampling. Tubers were counted and weighed, the numbers of stems were counted and the lengths of the individual stems were measured. For the detection of Cms by immunofluorescence (IF) cell staining, the tubers were washed with tap water and the heel end of each tuber was sampled in sterile 0.01 mol/l phosphate buffer pH 7.4. A 1 cm piece of the stem was also taken from the base and 30 cm above the base. Samples were stored at –20 °C prior to IF analysis.

In Denmark, the experiment was mainly carried out like in Finland. Tubers were planted on 15 May in 2000 and on 15 May in 2001. Stems and heel ends of the tubers were washed and sampled as described earlier (Finnish trial). Stolons, and roots were also sampled in 2001. Because of the slower development of plants in Denmark, plants were sampled at longer intervals (4–5 weeks) than in Finland.

2.2 Yield trial

Based on the results obtained in 2000 and 2001, a field experiment was set up to determine possible yield losses in susceptible cultivar 'Hansa' in 2002. Only the streptomycin-resistant Cms strain NCPPB 4053, which proved to be very infective in 2000 and 2001, was used in this experiment. Tubers were inoculated similarly as in earlier experiments and planted on 22 May. Tubers were planted in eight rows (four rows/treatment) so that every second row was planted with control tubers. During the season, plants were harvested twice. At the first time, 12 plants per treatment and at the second time, 40 plants per treatment were harvested. Tubers were counted and weighed, and tuber and stem samples were taken for the IF analysis as described earlier.

2.3 Detection of Cms by immunofluorescence cell staining

Detection of Cms was based on standard IF cell-staining (De Boer and Wieczorek 1984) using monoclonal antibodies (Mab 9A1, Agdia, USA) and fluorescein isothiocyanate (FITC)-labelled secondary antibodies (Agdia, USA). Plant material was weighed and then crushed with a hammer in plastic bags. Two millilitres of 0.01 mol/l phosphate buffer, pH 7.4, was added per gram of plant material (exact amount of buffer was adjusted according to the weight of plant material to obtain the final buffer (ml) to plant material (g) ratio 2 : 1) or a standard amount (5 or 10 ml) of buffer was added per a weighed sample (Denmark). After shaking 4 h at room temperature, plant debris was removed by centrifugation of the extracts at 180 × *g* for 10 min. The supernatant was taken directly for IF analysis or stored at –20 °C if analyzed later. Extracts were tested on multiwell slides undiluted and 10^{-1}, 10^{-2}, and 10^{-3} diluted if necessary. Samples (20 µl) were fixed with acetone on the slides. Slides were observed at magnification of 1000× under a microscope (Zeiss Axioscope, Germany or Microphot FXA, Nikon Co., Japan) equipped with an UV lamp and a filter set for fluorescein. From every sample, 60 microscope fields were observed using appropriate dilution and all stained bacteria of right size and shape were counted. Known positive samples and samples spiked with Cms were used as positive

controls. Negative controls were Cms-free potato extracts or buffer. The number of bacteria was calculated for a gram of fresh plant material extracted using the known buffer to plant material ratio in the extracts.

2.4 Data analysis

Statistical analyses were performed using SPSS software (SPSS Inc., USA, 2001). Means of yield measurements were compared by the independent samples t-test.

3 Results

3.1 Population development in Finland

The population development of Cms in potatoes grown in Finland was monitored by IF cell staining in 2 years, 2000 and 2001.

Inoculation of potato tubers with the fluidal Cms strain NCPPB 4053 resulted in higher densities of Cms in plants during the growing season than inoculation with the non-mucoid strain NCPPB 3898. NCPPB 3898 colonized potato plants of both cultivars ('Hansa' and 'Desiree') poorly reaching at maximum a density of 10^5–10^8 cells per gram of stem or tuber tissue (Fig. 1). The highest densities of the non-mucoid strain (10^7–10^8 cells g^{-1}) were found in 'Desiree' at the beginning of the season in 2000.

Population densities of the fluidal Cms strain (4053) increased rapidly in the beginning of the seasons, and apparently already before the first sampling times (Fig. 1). The population density reached its maximum 80–100 days from planting in 'Hansa'. Lower levels of Cms were found in tubers of the more resistant cultivar 'Desiree' than in those of the susceptible 'Hansa'. In stems, the bacterial densities did not differ between the two cultivars in 2001, whereas in 2000 the densities in 'Hansa' exceeded those in 'Desiree' during the first 80 days after planting. Stems of 'Desiree' were rapidly colonized by Cms in Finland (Fig. 1 and 3); high densities ($> 10^6$ cells g^{-1}) were found 30 cm above the base already 60 days after planting whereas in 'Hansa' lower densities of ca. 10^3–10^5 cells g^{-1} were found. In all cases, control plants remained free of Cms throughout the seasons.

In 'Hansa' inoculated with the Cms strain 4053, typical vascular symptoms began to develop in tubers at 80–90 days from planting in 2000 and 2001. The first symptoms were observed inside the tubers in the vascular ring as bacterial slime, but later in the season, the complete vascular systems were destroyed, and cracked, completely rotten tubers were found in many plants. Tubers without symptoms or with mild symptoms were also found. Stems started to wilt, and chlorosis and necrosis of some leaves were detectable after the third sampling time. At the time of symptoms became visible, average cell densities of strain 4053 ranged between 3×10^8 and 7×10^9 cells per g of tuber tissue in 2000 and 2001, respectively.

No visual symptoms were observed in the tolerant cultivar 'Desiree' at any sampling point in either stems or tubers independently of the strain used. Possibly, Cms (strain 4053) densities of 10^6 cells per gram of tuber tissue were insufficient to initiate symptom development in 'Desiree'. Densities of Cms strain 4053 in stems of 'Desiree', however, were not different from those in 'Hansa', but foliar symptoms were detected neither. No visual symptoms were observed in the Cms strain NCPPB 3898-inoculated plants in 'Hansa' as well as in 'Desiree'.

3.2 Population development in Denmark

In Denmark, the population development of both Cms strains was quite similar to the pattern found in Finland. The density of the non-mucoid strain 3898 in both cultivars did not reach even the level observed in Finland and most plants were negative when sampled and tested for Cms with IF cell staining (data not shown). The population of the fluidal strain 4053 followed the trend observed in Finland, although bacterial densities in stems and tubers of both cultivars were slightly lower in Denmark than in Finland particularly in 2001 (Fig. 2).

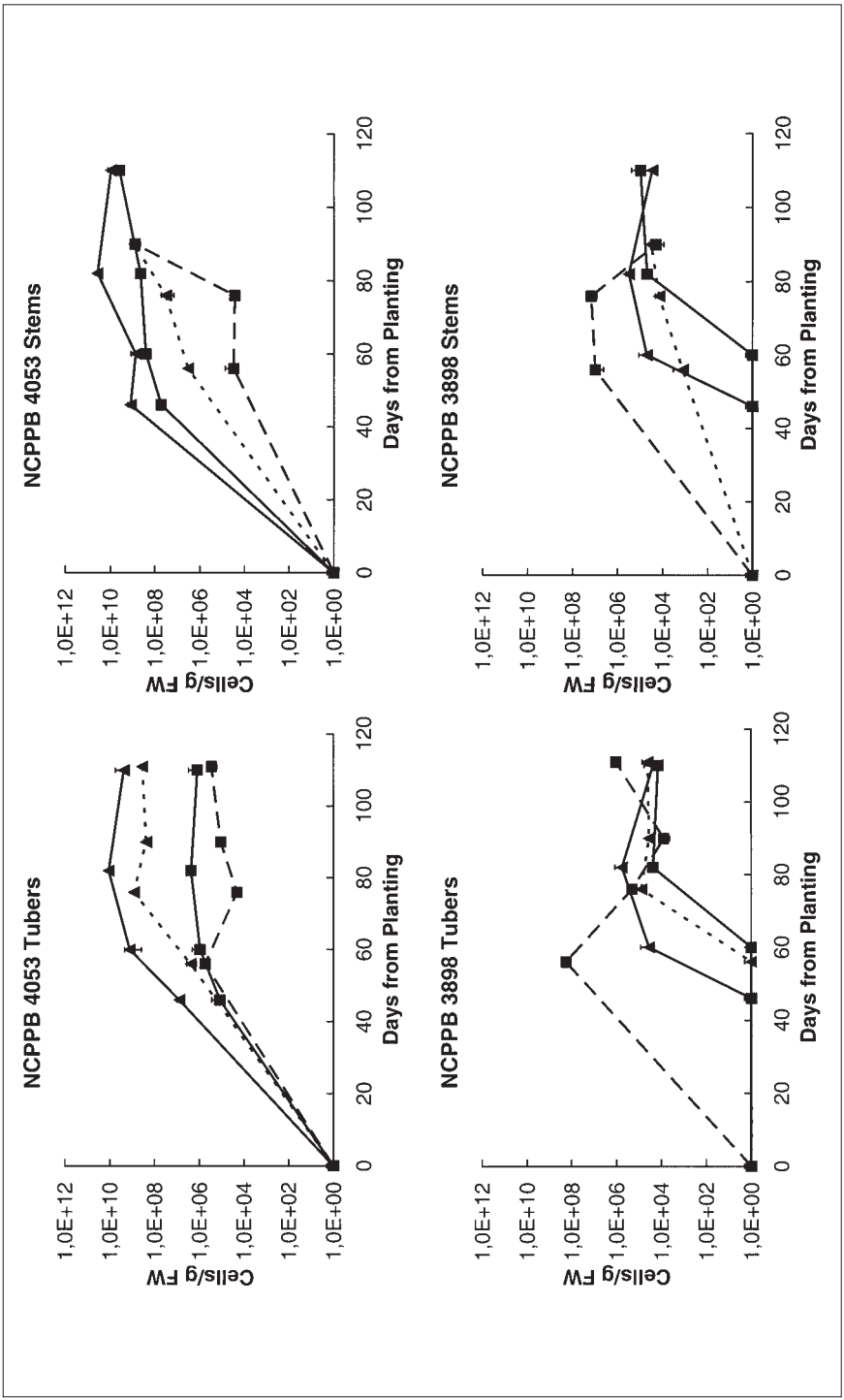

Fig. 1. The population development of fluidal streptomycin-resistant strain NCPPB 4053 (upper diagrams) and non-mucoid rifampicin-resistant strain NCPPB 3898 (lower diagrams) of *Clavibacter michiganensis* subsp. *sepedonicus* in tubers and stems (bases) of cultivars 'Hansa' and 'Desiree' under field conditions in Finland in 2000 and 2001. Symbols: Hansa 2000 - - ▲ - -, Hansa 2001 —▲—, Desiree 2000 - - ■ - -, Desiree 2001 —■—. The standard deviations for the means are shown with the bars.

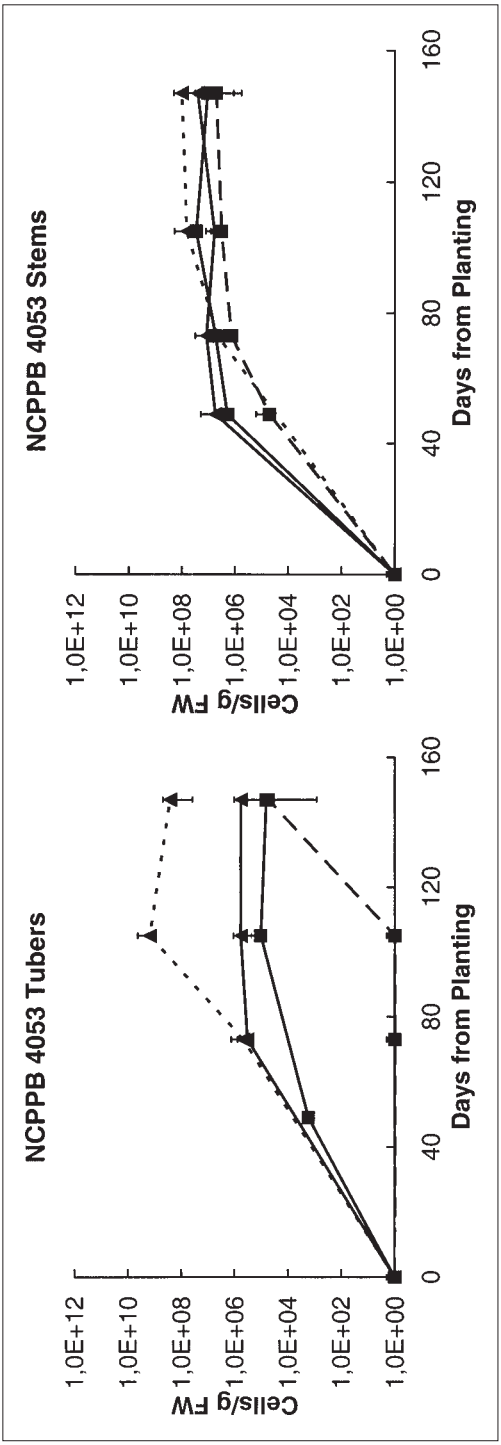

Fig. 2. The population development of fluidal streptomycin-resistant strain NCPPB 4053 of *Clavibacter michiganensis* subsp. *sepedonicus* in tubers and stems (bases) of cultivars 'Hansa' and 'Desiree' under field conditions in Denmark in 2000 and 2001. Symbols: Hansa 2000 - - ▲ - -, Hansa 2001 —▲—, Desiree 2000 - - ■ - -, Desiree 2001 —■—. The standard deviations for the means are shown with the bars.

In Denmark (2001), Cms population in tubers of 'Desiree' was at the same level as in 'Hansa'. In Finland, a clear difference between cultivars was observed (Fig. 1 and 2). In Denmark, Cms population densities increased more rapidly in lower and higher stem parts of 'Hansa' than of 'Desiree' (Fig. 1 and 3) that differs from observation made in Finland. Densities of Cms strain 4053 in roots of 'Hansa' were at the same level as in tubers when densities in stolons were closer to densities found in stems (Fig. 2 and 4). In 'Desiree', Cms concentration of stems exceeded those found in roots, stolons or tubers.

Fewer symptomatic plants were found in Denmark than in Finland in 2001, when the population density (strain 4053) was 10^6 cells per gram in tubers in Denmark. Even with the susceptible cultivar ('Hansa'), this low density could not induce symptom expression as observed with the more tolerant cultivar ('Desiree') in Finland. Population density was higher in Denmark in 2000 than in 2001 and more than 50 % the plants of 'Hansa' showed external symptoms (heavy infection) in tubers at the time of harvest. Foliar symptoms emerged in few stems in Denmark after 100 days from planting in both years.

3.3 Yield reduction caused by Cms infection

In Finland, severe infection with the Cms strain NCPPB 4053 reduced the yield of 'Hansa' (weight of tubers per plant) by 41–56 % on average in Cms-inoculated plants compared to the controls in 2002 (Table 1). The number of tubers, the total weight of tubers per plant and the weight per tuber were significantly ($P < 0.01$ or $P < 0.001$) lower in Cms-infected plants than in controls. The percentage reduction was greater in the average number of tubers than in the average weight per tuber. The tuber yield was affected already in early season

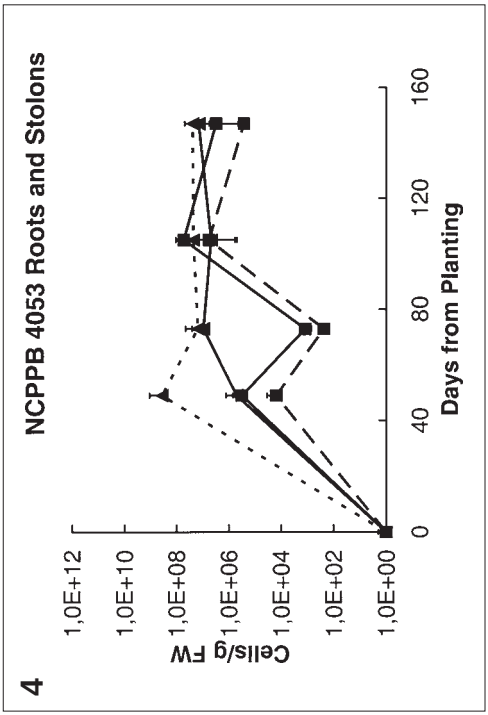

Fig. 3. The population development of Cms (streptomycin-resistant strain NCPPB 4053) in stems of 'Hansa' and 'Desiree' 30 cm above base in Finland and in Denmark in 2001. Symbols: Hansa Denmark - - ▲ - -, Hansa Finland —▲—, Desiree Denmark - - ■ - -, Desiree Finland —■—. The standard deviations for the means are shown with the bars.

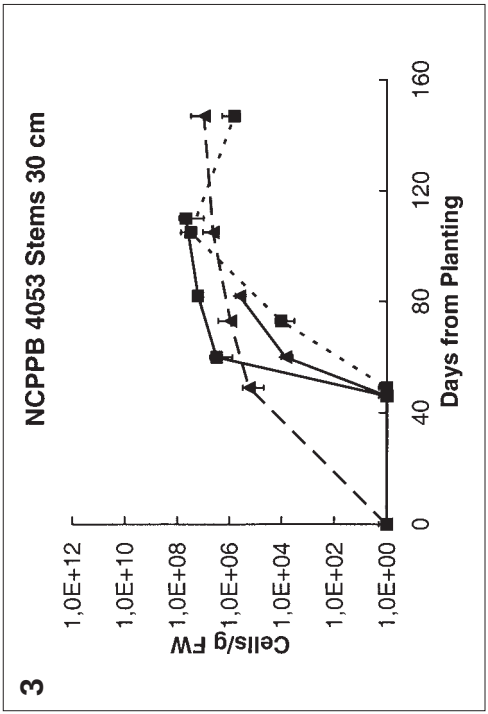

Fig. 4. The population development of Cms (streptomycin-resistant strain NCPPB 4053) in roots and stolons of 'Hansa' and 'Desiree' in Denmark in 2001. Symbols: Hansa stolons - - ▲ - -, Hansa roots —▲—, Desiree stolons - - ■ - -, Desiree roots —■—. The standard deviations for the means are shown with the bars.

Fig. 5. Monthly mean temperatures and precipitation registered at the research station of Jokioinen in Finland and at the research station of Slagelse in Denmark in 2000 and 2001. Line symbols for temperature: Finland 2000 —▲—, Finland 2001 —■—, Denmark 2000 - - ▲ - -, and Denmark 2001 - - ■ - -. Bar symbols for precipitation: Finland 2000 □, Finland 2001 ■, Denmark 2000 ■ and Denmark 2001 ■.

before any visible symptoms was detected. The number of stems was not affected by Cms, but in infected plants, the stems were significantly ($P < 0.01$ or $P < 0.05$) shorter at the end of season (Table 2). In the more tolerant cultivar 'Desiree', no significant reduction on yield or stem growth was observed either in 2000 or 2001 (data not shown).

4 Discussion

The population development of the fluidal and the non-mucoid (dry) Cms strain was studied in a tolerant ('Desiree') and a susceptible ('Hansa') cultivar under field conditions in Finland and Denmark. Inoculation with the fluidal Cms strain (NCPPB 4053) resulted in a higher density of bacteria in tubers and stems than inoculation with the non-mucoid strain (NCPPB 3898), suggesting that the ecological fitness between strains may be different. Only the fluidal, but not the non-mucoid strain induced disease symptoms in 'Hansa'. Fluidal and non-mucoid strains are known to differ in their composition of the extracellular polysaccharides (EPS) (Bishop et al. 1988; Westra and Slack 1992). Cms has four components of EPS, of which two fractions are not produced in non-mucoid strains, but a clear correlation to avirulence or weaker symptom expression compared to the fluidal strains has not been found. In this work, the population density of the non-mucoid strain NCPPB3898 was very low, and no symptom expression was observed. These results are in accordance with our observations on wild type and mutant strains of Cms (Toivonen et al. unpublished), where also the wild type non-mucoid strain (NCPPB 3898) showed a weaker HR reaction and lower cellulase activity than the fluidal strain (NCPPB 4053).

Table 1. Effects of ring rot infection (streptomycin-resistant strain NCPPB 4053) on potato yield in susceptible potato cultivar 'Hansa' in Finland in 2002. After 63 days from planting, 12 plants, and after 98 days, 40 control and inoculated plants were analyzed

Days[1)]	Variable	Unit	Control	Cms	t-test[2)]
63	Number of tubers	No./plant	18.8	10.6	***
98	Number of tubers	No./plant	21.1	13.6	***
63	Weight of tubers	g/plant	384.5	167.5	***
98	Weight of tubers	g/plant	572.4	339.7	***
63	Weight per tuber	g/tuber	21.0	15.6	*
98	Weight per tuber	g/tuber	28.4	24.3	**

1) Days after planting
2) Significance level: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant $P > 0.05$

Table 2. Effects of ring rot infection (streptomycin-resistant strain NCPPB 4053) on the growth of potato stems in susceptible cultivar 'Hansa' in Finland in 2000 and 2001. Five control and inoculated plants from every treatment were analyzed

Days[1)]	Year	Variable	Unit	Control	Cms	t-test[2)]
56	2000	Number of stems	No./plant	6.4	5.8	ns
90	2000	Number of stems	No./plant	5.2	5.8	ns
46	2001	Number of stems	No./plant	5.4	5.4	ns
82	2001	Number of stems	No./plant	8.0	6.0	ns
56	2000	Length of stems	cm/plant	63.1	53.5	**
90	2000	Length of stems	cm/plant	64.0	51.0	*
46	2001	Length of stems	cm/plant	64.0	53.6	*
82	2001	Length of stems	cm/plant	69.8	57.6	*

1) Days after planting
2) Significance level: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant $P > 0.05$

Maximum population density was found within 90 days after planting. This is in accordance with the previous experiments in Canada where highest densities of Cms were detected in non-symptomatic stems 80 days after planting (De Boer and McCann 1989). Stolons and lower stem parts of the plants were colonized earlier than tubers and higher stem parts during the growing season in 'Hansa'. In 'Desiree', stems were colonized more effectively than other plant organs. Generally, daughter tubers develop later during the season than stems or roots, but the late colonization of tubers may also be explained by the physical barrier known to exist between stolon and tuber in potato, which slows down the movement of Cms into daughter tubers.

The bacterial population density was lower in the tubers of the more resistant cultivar 'Desiree' than in those of the susceptible 'Hansa', as observed already in 1948 (Starr and Riedl 1948) and later by De Boer and McCann (1989). Interestingly, bacterial densities in stems were at a similar level in both cultivars. In higher stem parts (30 cm from the base), populations of Cms in 'Desiree' increased even faster and to a higher density than in 'Hansa' in Finland. However, no visual symptoms were found in 'Desiree' at any sampling points although symptoms were readily visible in the susceptible 'Hansa'. In previous studies, 'Desiree' has also been described as symptomless, but the Cms concentration in stems has varied in relation to the more susceptible cultivars (De Boer and McCann 1989; De Boer and McCann 1990). Our study suggests that the tolerance of 'Desiree' to Cms may be related to lower levels of bacteria in tuber tissue rather than to lower levels in stem tissue, but in other tolerant cultivars alternative mechanisms may exists. Our data reveal that the tolerant ('Desiree') cultivars may possess a considerable amount of Cms in potato stems without symptom expression. Therefore the extensive use of tolerant cultivars, which are completely symptomless, but may contain a high number of bacteria in stems or tubers, are of particular concern as they may pose potential undetected sources of inoculum for bacterial spread. Based on these results, effective management of the potato ring rot requires that special concern should be pointed towards healthy looking but potentially heavily contaminated stems of tolerant cultivars to avoid spreading of Cms to other fields during the farming practice or inspection of fields. Furthermore, the detection of low level of Cms in tolerant tubers requires the use of very sensitive diagnostic test methods.

The population density increased in similar pattern in the Finnish and Danish potato fields. In this study, we did not intend to compare absolute Cms concentrations between locations, but rather to see if the population development follows the same trend, and how it is related to the symptom expression. Based on our results with 'Desiree' and 'Hansa' in Denmark and in Finland, symptoms become visible in tubers and also in stems when bacterial population exceeds 10^7–10^8 cells per gram of tuber tissue. In general, many factors influence bacterial population development and symptom expression, such as temperature and other environmental conditions, and inoculum concentration (Bishop and Slack 1987; Nelson and Kozub 1987; Westra and Slack 1994; Kawchuk et al. 1998) so sorting out the single factor behind bacterial population development in different locations is a difficult task. In Denmark, the highest number of bacteria in 'Hansa' occurred in 2000, but in Finland, more bacteria were found in 2001. Temperatures did not differ between locations in the middle of the season in 2000 and 2001, whereas precipitation was lower in Denmark in 2000 and in Finland in 2001 (Fig. 5). Precipitation in Denmark, however, was lower than in Finland in both years and, therefore, apparently cannot explain the differences in the observed levels of Cms populations.

Our data shows that under suitable conditions, Cms population can increase rapidly in the susceptible potato cultivars and cause severe crop losses. In cv. 'Hansa', typical vascular symptoms were readily visible around 80–90 days after planting, followed by stem wilting and collapse in field experiments in Finland. Severe infection reduced yield by 41–56 %, and the disease was capable of affecting yield already in early season before visual symptoms emerged. In 'Desiree', no yield or growth reduction was observed despite of relatively high numbers of bacteria found in stems and tubers. Sletten (1985) found similar cultivar-dependent and symptom-independent yield reduction, and the yield loss was more due to lowered tuber number than lowered weight of single tubers supporting our results. The number of stems seems not to be markedly affected by Cms, but the length of stems has been reported to become shorter in Cms-inoculated plants in many studies (Sletten 1985; Bishop and Slack 1987; Westra and Slack 1994).

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