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Ring rot symptom development on potato cultivars and lines in southern Alberta*

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Summary

Plants were grown in the field from seed pieces of potato cultivars injected with ring rot bacteria (*Corynebacterium sepedonicum*). Symptoms were produced with both 18 and 180 colony forming units (cfu) on cvs Norchip and Red Pontiac and with 180 cfu on cv. Belrus plants. Tuber symptoms were detected in all cultivars except Belrus and Teton. Tuber progeny produced plants that developed top symptoms on all cultivars except those of cv. Nooksack, Russet Burbank, and Teton. Tuber progeny of the second crop produced both plants and tubers with symptoms developing only in the cv. Nooksack. In another 3-year experiment, variability in the disease response of selected cultivars and lines was examined following knife-inoculation of tuber seed with a high level of ring rot bacteria. Significant correlations between top and tuber symptoms were detected, but they were not high enough to make unnecessary the examination of both top and tuber symptoms in ring rot disease selection studies.

Introduction

Bacterial ring rot of potatoes caused by *Corynebacterium sepedonicum* (Spieck. and Kotth.) Skapt. and Burkh. (*Clavibacter michiganense* subsp. *sepedonicum* (Spieck. and Kotth.) Davis et al.) continues to be of concern in the potato-growing areas of North America. Although its incidence has declined in many locations, the potential remains for serious losses to occur if it is not detected and if measures to control it are not initiated. Any factors that interfere with symptom expression may have serious consequences, particularly in seed areas.

Air (Larson & Walker, 1941) and soil (Logsdon, 1967) temperatures affect symptom development, and potato viruses can mask typical symptoms (Nelson & Torfason, 1974) or influence the severity of atypical (dwarf-rosetting) symptoms (Nelson & Kozub, 1987). Nelson (1982) showed that small numbers of ring rot bacteria (3 – 300 colony forming units) incited symptomless or latent infection when inoculated into potato plants (cv. Russet Burbank) or seed pieces (Nelson, 1982). When grown at 15 °C, plants of cv. Russet Burbank inoculated with ring rot bacteria, and

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those grown from inoculated seed pieces, developed atypical dwarf-rosetting ring rot symptoms while those inoculated and grown at 21 °C developed typical wilting and chlorosis (Nelson & Kozub, 1987). Cultivars that are tolerant but not immune to the ring rot pathogen, and which sometimes may develop erratic or atypical symptoms, can serve as sources of contamination for desirable susceptible cultivars (Manzer & McKenzie, 1988). Any lines found to be tolerant to the pathogen in the Prairie Potato Breeding Program are not recommended for licensing in the Prairie Provinces.

The purpose of this investigation was mainly two-fold. First, to determine the effect of low inoculum levels on initial infection and symptom development in plants and tubers of selected potato cultivars and the effects in later years on disease development in plants grown from successive progeny. Second, to ascertain the best criteria for determining tolerance in potato cultivars and lines using higher levels of inoculum to induce variations in disease response and to determine if there was a relationship between top and tuber symptoms. A minor part of the investigation was to examine the effect of temperature on symptom expression of a few potato genotypes.

Materials and methods

Experiment 1. Seven cultivars, some with a known reaction to the pathogen (Table 1), were inoculated with low inoculum levels to see if any of them would develop symptomless infection. Highly virulent cells of *C. sepedonicum* were rinsed from cultures on modified Burkholder agar (Nelson & Semeniuk, 1964), the resultant suspensions serially diluted, and viable cell concentrations recorded as colony forming units (cfu) from plate counts. Two low concentrations of inoculum (18 and 180 cfu) were each used to inoculate 20 seed pieces of each cultivar (Nelson, 1982). The 40 treatments were planted in a randomized block design with four replicates in an Orthic Dark Brown Chernozemic irrigated soil at Lethbridge, Alberta, on May 8, 1986. Plots were single rows 1.5 m long and 1.2 m apart; each contained five seed pieces planted at 0.3 m intervals. Uninoculated seed pieces in two replications were planted as check treatments to ensure that parent material was free of ring rot bacteria. Weeds and insects were controlled as described by Nelson (1982). Symptoms were recorded throughout the growing season and the final top symptom data were recorded on August 25 and 26 when one stem was taken from each hill of the treatments. Smears from the lower part of these stems were subjected to Gram and indirect fluorescent staining (De Boer & McNaughton, 1986). Plants without ring rot symptoms were considered to be infected if in most microscope fields large numbers (100 or more) of short, Gram-positive pleomorphic rods were detected or if moderate to large numbers (50 – 100) of short, pleomorphic fluorescing rods were seen following indirect fluorescent antibody staining. The plots were harvested on September 23, 1986, and the tubers placed in a controlled environmental store (Nelson, 1982). During storage, tubers for planting in 1987, were taken from each treatment, including the controls, and indexed for freedom from potato leaf roll virus that could mask ring rot symptoms (Nelson & Torfason, 1974). Strict precautions were taken to prevent cross contamination of seed tubers from the different treatments at all stages of handling and storage.

Table 1. Infection with or without symptoms in plants of seven potato cultivars and symptoms in tubers of these plants developing in field plots at Lethbridge in 1986 from seed pieced injected with two different numbers of colony forming units (cfu) of ring rot bacteria, and in 1987 and 1988 from seed of the preceding year's crop.

Year[a]	Infection[b]	cfu	Cultivars						
			Norland	Nooksack	Norchip	Russet Burbank	Red Pontiac	Belrus	Teton
1986	% hills S	1	0	0	10.0 ± 6.7[c]	0	10.0 ± 6.7	0	0
		1	0	0	10.0 ± 6.7	0	20.0 ± 10.3	5.3 ± 5.1	0
	% hills NS	1	0	25.0 ± 21.7	15.0 ± 8.0	11.1 ± 7.4	5.0 ± 4.9	0	0
		1	5.0 ± 4.9	15.4 ± 10.0	10.0 ± 6.7	0	20.0 ± 10.3	5.3 ± 5.1	15.0 ± 8.0
	% tubers S	1	2.8 ± 1.9	0	17.8 ± 5.7	2.3 ± 2.3	6.8 ± 3.3	0	0
		1	0	3.4 ± 2.4	17.4 ± 4.1	0	23.8 ± 5.4	0	0
1987	% hills S	1	3.3 ± 3.3	0	3.3 ± 3.3	0	36.7 ± 8.8	3.3 ± 3.3	0
		1	0	0	50.0 ± 9.1	0	13.3 ± 6.2	0	0
	% hills NS	1	0	0	10 ± 5.5	0	6.7 ± 4.6	0	0
		1	0	16.7 ± 6.8	23.3 ± 7.7	0	3.3 ± 3.3	0	0
	% tubers S	1	0.3 ± 0.3	0	3.7 ± 2.0	0	7.9 ± 2.6	0.6 ± 0.6	0
		1	0	10.2 ± 5.3	28.9 ± 7.0	0	8.6 ± 3.3	0	0

[a] In 1988, only cv. Nooksack showed any symptoms or detectable infection.
[b] S = symptoms NS = no symptoms; infection determined by Gram and indirect fluorescent antibody staining of smears from the lower stem.
[c] Observed percentage and standard errors for values >0%.

On May 6, 1987, a single sterilized knife was used to cut longitudinally all of the non-indexed tubers of a single treatment and then used to cut the indexed tubers of this treatment into seed pieces. The non-indexed tubers were discarded and the indexed seed pieces planted. A separate sterilized knife was used for each treatment. This cutting procedure was done to simulate commercial handling of seed tubers. The number of tubers with ring rot symptoms within each treatment was recorded as soon as all the tubers of that treatment had been cut. Indexed seed pieces were planted in a randomized block design as in the 1986 experiment, except that there were five replicates with rows 1.8 m long each containing six seed pieces. Control seed was planted in two replicates. The final records of symptoms were made on August 31, 1987, when one stem was taken from each hill of each treatment for smearing and staining as in 1986. Tubers were harvested on September 15 – 17, 1987, and stored and indexed as in the previous year. Tubers of cvs Norchip and Red Pontiac were not stored in 1987 but were scored for ring rot symptoms in the field.

On May 9, 1988, the indexed tubers harvested and stored in 1987 were cut into seed pieces and planted as in 1987. The dimensions of the plots were the same as in 1987 except that the treatments were replicated six times. Symptoms were recorded on August 29, 1988, and stems collected as before for smearing and staining. On September 8, 1988, the plots were harvested and the numbers of tubers with symptoms recorded in the field.

Experiment 2. Potato cultivars and advanced lines from various parts of the United States and Canada, and which formed part of the Prairie Potato Breeding Program, were inoculated with higher concentrations of the ring rot pathogen than in Experiment 1 to determine their response to infection. During 3 years of testing (1986 – 1988), certain lines were examined for only 1 year since other criteria indicated they were not suitable for culture in southern Alberta. However, three cultivars (Norland, Norking, and Teton) with varying resistance to the pathogen were tested each year.

Seed tubers were inoculated with ring rot bacteria by dipping a knife in a concentrated bacterial suspension ($2X10^9$ cfu ml^{-1}) prior to cutting each tuber; also the tissue around the eyes was pierced with the knife. Inoculated and uninoculated control seed pieces for each of the cultivars and lines were planted in a randomized block design in an Orthic Black Chernozemic dryland soil at Stavely, Alberta. Precautions were taken to prevent contamination of control seed. Treatments were replicated four times. Plots were single rows 1.8 m long spaced 1.2 m apart and each contained five seed pieces planted at 0.3 m intervals. Plot dimensions and treatment replications were the same in each of the 3 years of testing.

Each year ring rot top severity ratings (Nelson & Kozub, 1987) were recorded throughout the growing season, the last record being made before the onset of senescence. From 1 to 4 weeks after the final observations, symptoms in all tubers were also recorded. Neither stem nor tuber extracts were examined.

A few cultivars suspected of reacting differently to *C. sepedonicum* were selected from this investigation and, along with cv. Russet Norkotah selected from another study, were tested in controlled environments at 15 and 21 °C as described by Nelson & Kozub (1987).

Statistical analysis. In Experiment 1, for each year of testing, analyses of variance (Steel & Torrie, 1980) were carried out on the final percent of infected hills with ring rot symptoms, percent of infected hills with no symptoms, and the percent of tubers with symptoms, to determine if there were effects due to the interaction of cultivar and inoculum concentration and of their effects singly. Cultivars with no disease response were omitted from the analysis; logarithmic transformation was applied before analysis when it was evident that the magnitudes of the means and standard deviations were not independent. To simplify interpretation, estimates of disease responses are presented using untransformed data.

In Experiment 2, for each year of testing, analyses of variance for the randomized block design (Steel & Torrie, 1980) were carried out to compare the cultivars and lines for variation in their final percent hills with ring rot symptoms, final ring rot top severity rating and percent tubers with symptoms. Lines or cultivars with none or 100% symptoms for all replicates were excluded from the analyses and the data transformed where appropriate. Using the mean values for lines and cultivars, correlations were calculated to determine the relation between top and tuber symptoms.

Results

Experiment 1. Plants and progeny tubers of cvs Norchip and Red Pontiac grown from seed pieces injected in 1986 with 18 or 180 cfu of ring rot bacteria developed

ring rot symptoms, whereas only those seed pieces of cv. Belrus that had been injected with 180 cfu produced plants with symptoms but their tubers were found not to have symptoms (Table 1). For cultivars that were infected and had top symptoms, there were no significant differences among cultivars and inoculation treatments. At one or both inoculum concentrations, seed pieces of all cultivars generated infected plants without symptoms, but there were no significant effects of cultivar or inoculation treatment. Only two cultivars, Belrus and Teton, had no tuber symptoms in 1986. There were significant ($P<0.05$) differences among the remaining cultivars, with Norchip and Red Pontiac having the greatest percentage of tuber symptoms in 1986, but there was no effect of inoculum concentration or interaction with cultivars.

When tubers from the treatments of the 1986 crop were planted in 1987, top symptoms developed only on plants of cvs Belrus, Norchip, Norland and Red Pontiac (as in 1986, Table 1). As in 1986, cvs Norchip and Red Pontiac had the highest levels of plants with symptoms, but the original higher inoculation level of 180 cfu did not necessarily contribute to the highest number of infected plants with symptoms as indicated by the significant cultivar by inoculation treatment interaction. In 1987, symptomless infection was detected only in cvs Nooksack, Norchip and Red Pontiac, the cultivars that had the highest levels of symptomless infection in 1986. The greatest degree of symptomless infection in cvs Nooksack and Norchip corresponded to the highest original cfu concentration. Of the tubers harvested in 1987, all but those of cvs Russet Burbank and Teton showed symptoms of ring rot. As in 1986, tuber symptoms were greatest in cvs Norchip and Red Pontiac, although the effect of the original inoculum concentration was inconsistent. This inconsistency was even more pronounced when assessed by the extent of symptom development in tubers of cvs Norchip and Nooksack.

The 1987 seed tubers of cvs Norchip and Red Pontiac were not planted in 1988. Of the plants grown from the 1987 tubers, only those of cv. Nooksack derived from the original 180 cfu treatment showed both top and tuber symptoms or symptomless infection (33.2 ± 7.9% top symptoms; 37.2 ± 8.2% top infection with no symptoms and 23.3 ± 3.1% tuber symptoms). During 3 years of testing, no infection or symptoms were detected in any of the control plants or tubers.

Experiment 2. No attempt was made to rank all of the cultivars or lines into defined ring rot-resistant categories because many of them had been tested for only 1 year. However, the data can be used to show the variation in disease response for cultivars and lines within and over years, and to assess how closely top and tuber symptoms are related.

When high levels of inoculum were used, there was a wide range in mean symptom severity and percentage of hills with top symptoms for the cultivars and lines in each year (Table 2). Of the three cultivars used in all 3 years, the mean symptom severity and percent hills with symptoms varied little over the years. Cultivar Norland showed a high, Norking an intermediate, and Teton a very low response to infection. For the 2 years in which they were tested, the lines F76080, F77001 and W760 varied little in their response to infection but cvs Sangre and Banana were more variable. The correlation for all 3 years between the mean top severity rating and percentage hills with symptoms was $r=0.88$ ($P<0.01$), which indicated a marked relationship between test variables.

As with the top severity and symptom data, there was a wide variation between

Table 2. Mean[a] ring rot symptom severity[b] on plants of potato cultivars and lines grown in field plots at Stavely from seed pieces inoculated with ring rot bacteria, 1986 – 1988.

1986		1987		1988	
Norland[c]	4.15 (100)	*Norland*	4.05 (100)	*Norland*	3.90 (100)
Norking	2.10* (75 ± 10)	*Norking*	1.15* (70 ± 10)	*Norking*	1.45* (70 ± 10)
Teton	0.00* (0)	*Teton*	0.05* (5 ± 5)	*Teton*	0.00* (0)
Norchip	4.25 (100)	Mn9632	4.50 (100)	NDA398-1	4.40 (100)
Mn10162	4.65 (100)	Wis75-15	4.30 (100)	Hilite Russet	4.35 (100)
NDA8694-3	4.90 (100)	*F76080*	2.68* (83 ± 9)	*F76080*	2.80* (80 ± 9)
A7534-3	3.25 (100)	Wis74-7	3.95 (100)	Wis75-30	3.85 (100)
Red Pontiac	3.70 (100)	*Sangre*	2.15* (90 ± 7)	*Sangre*	3.60 (100)
Russet Burbank	1.50* (55 ± 11)	W847	3.15* (100)	A7411-2	2.05* (80 ± 9)
Niska (W848)	3.85 (100)	*F77001*	2.65* (95 ± 5)	*F77001*	2.33* (90 ± 7)
A68710-5	3.10 (100)	*Banana*	1.15* (80 ± 9)	*Banana*	2.40* (95 ± 5)
A74117-9	3.45 (100)			ND383-9	3.95 (90 ± 7)
W760	3.15 (90 ± 7)			*W760*	3.48 (90 ± 7)
Nooksack	0.53* (24 ± 10)				
SE[d]	0.38		0.26		0.34

* Significantly different ($P < 0.05$) from Norland within a year according to the LSD test; Teton excluded from the analysis.
[a] Means of four replications for each test year.
[b] Symptom severity rated on a 0 to 5 scale; 0 = no symptoms, 5 = plant dead. Percent hills with symptoms ± standard error given in brackets.
[c] Italics are used for cultivars or lines that underwent 2 or more years of testing.
[d] SE = standard error of a severity mean with 36, 27 and 33 df for 1986, 1987 and 1988 respectively.

lines and cultivars within each year in the percentage of tubers with ring rot symptoms (Table 3). Similar ranges of 0 – 70, 0.6 – 59 and 0 – 60 were evident in 1986, 1987 and 1988, respectively. For the cultivars that were used in all 3 years, Teton showed little disease response in all years, whereas the range of means for cvs Norland and Norking was more than 20%. The range was smaller in the cultivars and lines F76080, Sangre, F77001, Banana and W760 in the 2 years they were tested.

In 1986, tubers of cvs Norland and Norking had significantly lower levels of symptoms than several other lines and cultivars (Table 3). For cv. Norland, this result is not in agreement with its top severity ranking relative to other lines and cultivars in the 3 years of testing (Table 2). In 1987 and 1988, cultivars and lines that had significantly lower percentages of tuber symptoms than cv. Norland generally had significantly lower top severity ratings as well; accordingly, there appears to be some agreement between top and tuber symptoms for these years. However, the levels of ring rot were the same for cvs Norland and Norking in 1986 and 1988 while cv. Norking had significantly lower top severity ratings than cv. Norland in all 3 years.

The correlations between the percentage of tubers with ring rot and the percentage of hills with top symptoms was 0.55 ($P < 0.01$), while the correlation between the

Table 3. Mean[a] percentage of tubers with ring rot symptoms from plants of potato cultivars grown in field plots at Stavely from seed pieces inoculated with ring rot bacteria, 1986 – 1988.

1986		1987		1988	
Norland[b]	12.3	*Norland*	37.6	*Norland*	34.3
Norking	12.2	*Norking*	18.9*	*Norking*	33.2
Teton	0.0	*Teton*	0.6*	*Teton*	0.0
Norchip	70.2*	Mn9632	58.9*	NDA398-1	60.1*
Mn10162	63.4*	Wis75-17	53.9	Hilite Russet	55.1*
NDA8694-3	49.6*	*F76080*	48.6	*F76080*	38.3
A7534-3	41.3*	Wis74-7	46.2	Wis75-30	50.4*
Red Pontiac	40.7*	*Sangre*	40.9	*Sangre*	47.2
Russet Burbank	39.5*	W847	34.7	A7411-2	48.0
Niska (W848)	38.3*	*F77001*	12.1*	*F77001*	4.6*
A68710-5	33.2*	*Banana*	3.3*	*Banana*	8.9*
A74117-9	28.7			ND383-9	8.6*
W760	23.2			*W760*	31.2
Nooksack	20.5				
SE[c]	6.7		6.2		5.5

* Significantly different ($P < 0.05$) from Norland within a year according to the LSD test; Teton excluded from the analysis.
[a] Mean of four replications for each test year.
[b] Italics are used for cultivars or lines that underwent 2 or more years of testing.
[c] SE = standard error of a mean with 36, 27 and 33 df for 1986, 1987 and 1988, respectively.

percentage of tubers with symptoms and the top severity rating was 0.69 ($P < 0.01$). Thus, there was some relation between top and tuber symptoms.

Symptom development in a controlled environment. A few cultivars suspected of reacting differently to the ring rot pathogen at 15 °C (Nelson & Kozub, 1987) were examined in growth cabinets. The cv. Banana infected with *C. sepedonicum* and grown at Stavely in 1988 showed some atypical, dwarf-rosetting symptoms; however, it was not tested in the growth cabinets.

Stem cuttings of the cv. Russet Norkotah, root-inoculated with ring rot bacteria and grown at 15 °C, developed both typical chlorotic and wilting as well as atypical dwarf-rosetting symptoms that persisted 7 weeks after inoculation (Fig. 1A and 1B). At 21 °C, inoculated cv. Russet Norkotah plants exhibited very upright, narrow, stunted growth with typical ring rot symptoms (Fig. 1C).

Plants of cv. Nooksack, infected with *C. sepedonicum* and grown at 15 °C, were bunchy and dwarfed but without dwarf-rosetting (Fig. 1D). Seven weeks after inoculation, a few cv. Nooksack plants had marginal leaf necrosis but no wilt symptoms.

The growth of infected cv. Red Pontiac plants at 15 °C was stunted and they had typical ring rot symptoms (Fig. 1E). When Nelson & Kozub (1983), inoculated plants of this cultivar and grew them at 21 °C under light conditions similar to those employed here (Nelson & Kozub, 1987), they developed typical ring rot symptoms without stunting.

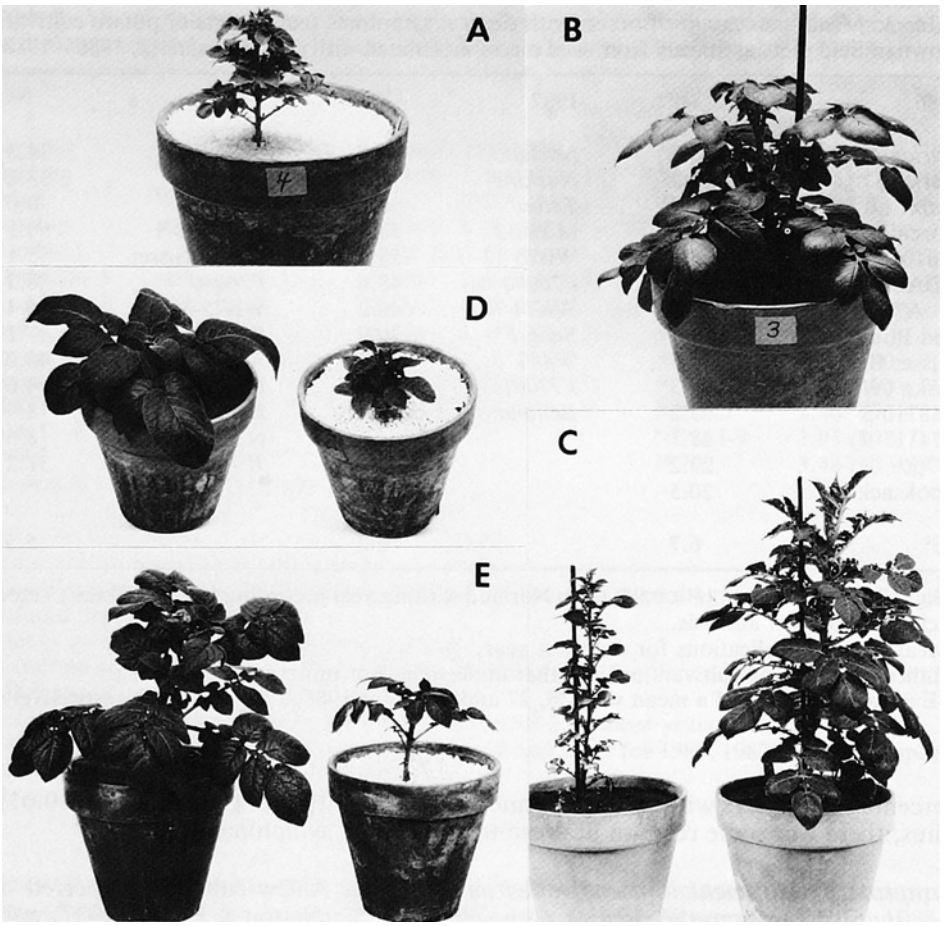

Fig. 1. Ring rot symptoms on plants of different potato cultivars grown at 15 °C or 21 °C. (A) cv. Russet Norkotah, at 15 °C with atypical and typical ring rot symptoms 4 weeks after inoculation; (B) cv. Russet Norkotah control plant at 15 °C; (C) cv. Russet Norkotah at 21 °C; left, typical ring rot plant symptoms: right, control plant 5 weeks after inoculation; (D) cv. Nooksack at 15 °C; left, control plant, right showing stunting from ring rot 7 weeks after inoculation; (E) cv. Red Pontiac at 15 °C; left, control plant, right, stunting with typical ring rot symptoms 4 weeks after inoculation.

Discussion

Seed pieces of cv. Russet Burbank, inoculated with two low levels of ring rot bacteria, produced plants with symptomless infection, as in a previous study by Nelson (1982); however, unlike that previous study, continued propagation with

tubers from this latently infected crop resulted in no discernible infection or symptom development over the next 2 years. That previous investigation, and one other by Manzer & McKenzie (1988), did not place this cultivar in a highly resistant category, although latent infection is known to persist in it for long periods (S.H. De Boer and G.A. Secor, personal communications). Infection and symptom development did not increase after the first year in the cvs Belrus, Norland and Teton; this is not surprising since cvs Belrus and Teton are reputed to be resistant (Manzer & McKenzie, 1988). However, the results obtained with cv. Norland were not expected since it was found to be highly susceptible in Experiment 2 of this study and elsewhere (Manzer & McKenzie, 1988). Under southern Alberta conditions, the cvs Norchip and Red Pontiac proved to be highly susceptible, as they had high levels of top and tuber symptoms in the 2 years that they were grown; however, levels of symptoms were maintained rather than increased from the first to the second year. While cv. Nooksack did not have any top symptoms in the first 2 years it was grown, it proved to be an exception. Tuber symptoms increased steadily to the third year and top infection with and without symptoms increased significantly in the third year. Why levels of infection and symptoms were not maintained or disappeared altogether in several of the cultivars examined in Experiment 1 is difficult to understand. Sampling procedures were similar to those in the previous study (Nelson, 1982); however, the field plots where the tests were carried out had been used for ring rot studies over a 10-year period and antagonism to the pathogen could have built up. Moreover, extensive, extreme soil drifting prior to planting in 1987 and very high field temperatures for intermittent periods in the summers might have affected the pathogen's development. However, none of these factors altered the manifestation of the disease in the cvs Nooksack, Norchip or Red Pontiac. While such results can and do occur, they are difficult to explain.

All of the cultivars and lines could not be ranked into defined resistance groups in Experiment 2 because many were tested for only 1 year. However, we were able to show that our inoculation methods were satisfactory, as top symptom levels were high in many lines and cultivars and resulted in high, intermediate and low response categories to infection by the pathogen in 3 years of testing. Because there appeared to be some relationship between top and tuber symptoms, we had hoped initially that only top observations would be necessary to determine resistance. However, while correlations between top severity and tuber symptom data were significant in 3 years of testing, they were not high enough to allow precise prediction of tuber symptoms from top symptoms. Further evidence of this lack of predictability was shown by a few lines that developed severe top symptoms in response to infection and which did not show high levels or severe tuber symptoms. These results show that examination of both top and tuber symptoms will be necessary in determining resistance to the ring rot pathogen.

The importance of controlled environment studies when determining responses to infection by the ring rot pathogen was demonstrated in this investigation. Dwarf-rosetting, bunchiness and stunting observed on different cultivars grown at 15 °C suggest that these symptoms might appear on these cultivars in the field during years when mean air temperatures are lower than normal (Nelson & Howard, 1982).

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