Solke H. De Boer
Agriculture Canada Research Station, Vancouver, BC

Steven A. Slack
University of Wisconsin-Madison

Current Status and Prospects for Detecting and Controlling Bacterial Ring Rot of Potatoes in North America

Bacterial ring rot (BRR) is the most feared potato disease in North America. Commercial production (comprised of seed, fresh, and processing industries) of potatoes is by vegetative propagation of tubers (enlarged, fleshy, underground stems) called seed tubers. Because the disease-causing bacterium is spread rapidly from tuber to tuber in planting operations and because all tubers from infected plants may rot, an entire crop can be lost.

For more than four decades, the primary control procedure has been for all certifying agencies in North America to reject potato lots for seed usage following the detection of even one diseased plant in a field or one tuber in a lot (Zero Tolerance regulation). This strict regulatory approach has been effective in limiting the disease to low frequencies both in and among all potato production operations but not in eliminating the disease from potato production areas. Unfortunately, BRR has become regarded as a problem and responsibility solely of the seed potato industry. Because individual seed growers may suffer both socially and economically when BRR is detected or even suspected in their seed stocks, an aura of secrecy surrounds BRR detection. Inoculum sources are often impossible to determine.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1984 The American Phytopathological Society

Historical Perspective

BRR, first described by Appel in Germany in 1906, was first detected in North America in 1931, in the Province of Quebec. The next year it was found in the state of Maine and by 1940 had been reported in all the major potato-growing states and provinces of the United States and Canada.

The causal organism, described by Spieckermann in 1910, is the bacterium *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh. (Fig. 1). Under field conditions, this prokaryotic microorganism is known to cause disease only in potatoes, but on inoculation it can

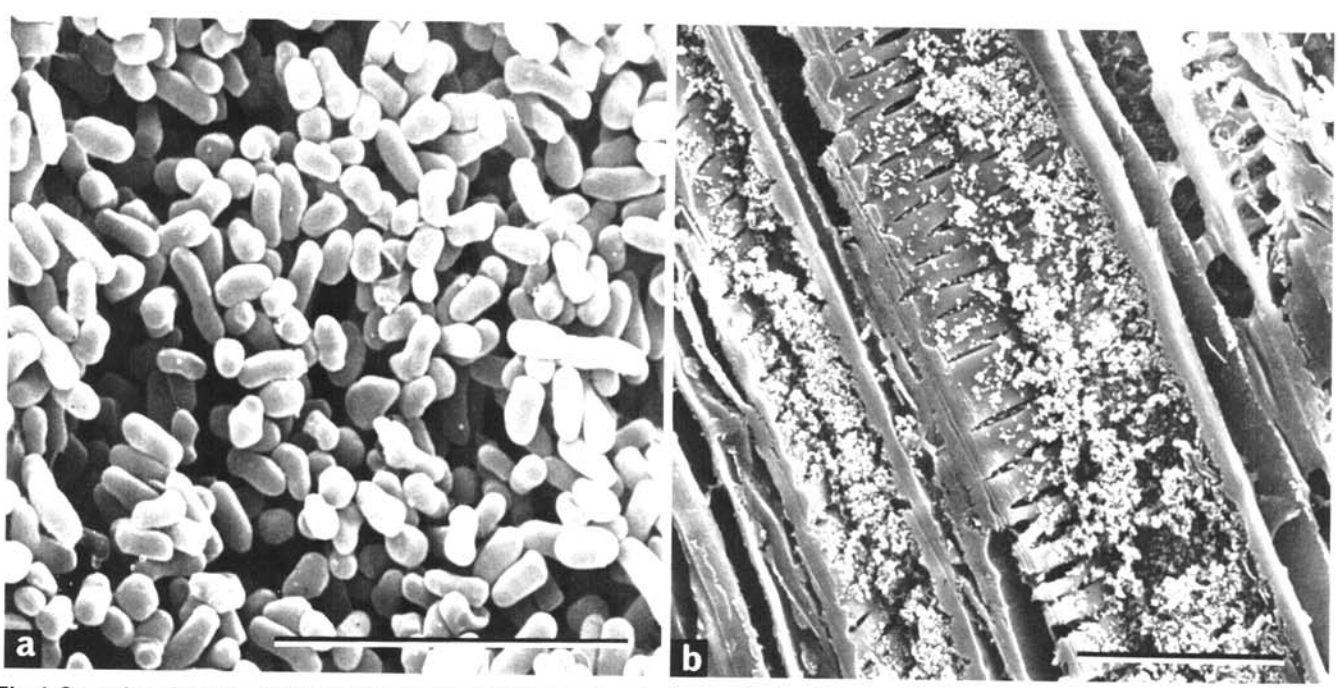


Fig. 1. Scanning electron micrographs of *Corynebacterium sepedonicum* cells in xylem vessels of a potato stem. (A) High magnification shows individual bacterial cell morphologies (bar = 5 μm) and (B) low magnification shows large masses of cells in two adjacent xylem vessels (bar = 50 μm).

infect other plants such as tomato and eggplant. It is morphologically and biochemically similar to other gram-positive plant-pathogenic bacteria such as *C. michiganense* and *C. insidiosum*, the causal agents of bacterial canker of tomato and alfalfa bacterial wilt, respectively, and is related to the poorly defined group of coryneform bacteria commonly found in soil. Although this article is not a taxonomic treatment of the genus *Corynebacterium*, we note that the phytopathogenic coryneforms placed in this genus form a heterogeneous group and that changes in existing generic and species designations have recently been proposed (14).

C. sepedonicum is characterized by a slow growth rate, requiring 5–10 days for individual colonies to become visible on agar media. It has a requirement for several growth factors. These characteristics make it a difficult organism to isolate and study, which probably accounts for the paucity of biochemical and physiological data on this bacterium 74 years after the first description.

Research and field observations made during the late 1930s and early 1940s indicated that the chief source of inoculum for BRR was infected seed tubers and that the bacterium spread mainly during the cutting of seed tubers or during other operations (eg, pick-type planters and root pruning by cultivation) that injured tubers or plants (1,7,13). If harvested tubers from a crop with only one or a few plants showing BRR symptoms were replanted, over 50% of the plants in the next crop might develop symptoms, with a concomitant loss in tuber yield. Hence, many procedures for disinfecting cutting knives, machinery, storage areas, and seed potato carriers were published in research journals and recommended in extension bulletins. Greatest emphasis, however, was placed on using seed free from BRR. The disease could be eliminated from a farm by disposing of all potatoes, then disinfecting all equipment and storage areas and introducing seed free from BRR. If the disease could thus be eliminated from a farm, it was reasoned that it could also be eliminated from a state, country, or continent. This was the concept that led to adoption of the Zero Tolerance rule for all classes of certified seed potatoes in North America.

Zero Tolerance

In potato certification programs, tolerance levels are set for various diseases for each class of seed (*classes* are determined by the strictness of the disease tolerance level) (10). If a disease exceeds the tolerance level for a given class of seed, the seed lot is either lowered to the next acceptable seed class or rejected from use as certified seed stock. In the early 1940s, the tolerance level for BRR was set at zero for all classes of seed potatoes because the bacterium had been shown to spread rapidly within a contaminated seed lot and because eradication of the recently introduced bacterium was desired. Zero Tolerance means that if a single plant in a field or a single tuber in a seed lot is infected with BRR, the seed lot cannot be certified. It can be sold as commercial table stock, processed, or destroyed.

A grower whose seed lot is not certified because of BRR may lose several thousand dollars immediately and have a severely damaged reputation for several years. The consequences of otherwise negligible levels of ring rot are why growers fear the disease the most and why some are reluctant to tell authorities they suspect or may have found the disease in their crops. Cooperation is often difficult to obtain in subsequent investigations to determine the initial source and the extent of contamination on a farm. Even under the most cooperative conditions, determining the inoculum source is sometimes impossible.

Symptoms and Diagnosis

The implications of a positive ring rot diagnosis have placed a great responsibility on inspectors and plant pathologists to ensure the diagnosis is correct. Traditionally, diagnosis has depended primarily on symptom expression.

In the early stages of symptom development on growing plants, wilted leaves are slightly rolled at the margins and light green to pale yellow areas develop in the interveinal spaces (Fig. 2). As wilt progresses, the affected foliage becomes necrotic, beginning at the leaf margins, and entire stems may collapse. Foliar symptoms usually do not appear until after flowering in midseason to late season, but certain cultivars (eg, Russet Burbank) may also develop an early-season dwarf-rosette symptom. When infected stems are cut transversely near the base, a milky exudate generally can be squeezed from the cut end. Tubers develop a characteristic "ring rot" symptom visible in transverse sections as a breakdown in the vascular ring (Fig. 3A). In the early stages of symptom development, affected tuber tissue is creamy yellow and cheesy in texture. The rot is odorless and confined to the vascular ring. Later, the vascular ring may become brown and necrotic and the rot may spread throughout the tuber, especially when secondary organisms proliferate in the diseased tissue. Surface cracks and dark blotches immediately beneath the periderm (Fig. 3B) are signs of advanced decay.

Although ring rot diagnosis is based primarily on the characteristic symptoms, diagnosis is normally confirmed by a laboratory test (6). Until recently, the usual confirmatory test was to Gram stain smears prepared from stem

Fig. 2. Initial bacterial ring rot symptoms of interveinal chlorosis and marginal necrosis of cupped leaves.

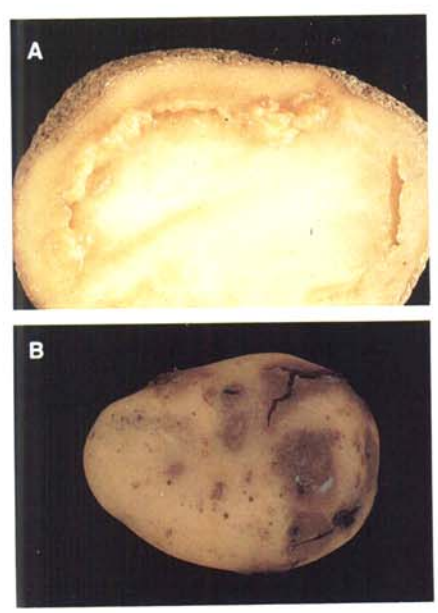


Fig. 3. Potato tubers with (A) characteristic "ring rot" or vascular ring breakdown and (B) external periderm cracking associated with advanced stages of decay.

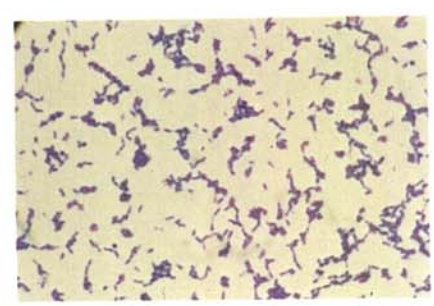


Fig. 4. *Corynebacterium sepedonicum* showing a gram-positive stain reaction (×500).

exudates or tuber vascular tissue. Positive BRR preparations contain many gram-positive bacteria (0.5 × 1.0 μm), some of which are slightly club-shaped and appear in L or V formations (Fig. 4). When symptoms are typical and Gram stains are prepared and interpreted carefully, these diagnostic procedures appear to work satisfactorily. However, when symptoms are atypical or masked by advanced decay from secondary microorganisms, as happens frequently, diagnosis is difficult. Since secondary microorganisms can include other gram-positive bacteria (eg, *Clostridium* spp., *Bacillus* spp., and saprophytic coryneforms), results of the confirmatory Gram stain test may be ambiguous. Serological tests have considerably improved diagnostic accuracy but have not eliminated the need for a specific diagnostic test.

Table 1. Bactericides useful for disinfecting equipment and storage areas

Disinfectant[a]	Concentration (a.i.)	Inactivated by organic matter	Comments
Chlorine	0.5%	+	Corrosive to metals; addition of detergent increases effectiveness; respiratory irritant
Formaldehyde	2–5%	−	Not very effective in cold temperatures; corrosive; irritating odor
Iodine	Follow container instructions	+	Corrosive
Phenolic compounds	1–3% (or follow container instructions)	−	May leave gummy residue
Quaternary ammonium compounds	0.08% (or follow container instructions)	+	Odorless, nonstaining, noncorrosive, stable; effective only on long exposure
Steam	. . .	−	Requires adequate time for heating surfaces

[a]Consult local authorities for guidelines on permitted usage with food crops.

Why Does BRR Persist?

The inherent inaccuracies of diagnostic procedures have undoubtedly had a bearing on the consistency with which the Zero Tolerance regulation could be implemented. Nevertheless, the Zero Tolerance regulation can be credited with reducing BRR incidence among and within seed lots from the levels observed in the 1930s and 1940s and with maintaining the incidence at a generally low level. Although periodic BRR outbreaks still occur in North America, the disease no longer occurs in several European countries, including Germany, where ring rot was first recognized. Successful eradication has been attributed mainly to the use of whole seed and to the avoidance of planters that use spikes to place individual seed pieces into furrows (pick-type planters). The North American industry has not eliminated cutting of seed tubers because tubers of North American potato cultivars are larger than those of European cultivars and cut seed, on a weight basis, plants a larger acreage than does whole seed. Pick-type planters continue to be used because they are the most efficient, ie, plant density is greater because in-row skips are fewer.

Commercial production practices have enhanced dissemination of BRR in the United States and Canada. Community seed cutting in which one organization cuts seed tubers for several farms especially increases the probability of a BRR-contaminated seed lot contaminating several other seed lots and farms. Community vehicles that carry seed, fresh, and processing potatoes for several farms also increase the probability of BRR spread. These practices, combined with inadequate sanitation procedures, are an invitation to destructive crop losses.

Effective sanitation requires both cleaning and disinfecting of all surfaces that may come in contact with seed tubers (Table 1). *C. sepedonicum* cells are resistant to drying and can survive quite well in soil and plant debris dried onto machinery, equipment, storage and vehicle walls and floors, etc.

Undetected spread and maintenance of BRR in seed lots have been sources of concern, because *C. sepedonicum* may be present in plants or tubers that do not show symptoms. Low inoculum levels, late-season infection, and environmental (eg, cool and wet) conditions that suppress or mask symptom expression have been associated with symptomless infections. Symptomless stems and tubers may support up to 10^9 and 10^7 cfu of bacteria per gram of tissue, respectively (A. L. Bishop and S. A. Slack, *unpublished*). The extent to which symptomless infections may be maintained from season to season in seed lots and to which they serve as inoculum for BRR-free stock is not known. Further, the mechanisms governing pathogenesis are poorly understood.

There are perhaps additional reasons why BRR has not been eradicated by regulation alone. Possibly there are unknown sources of inoculum from which BRR-free planting material can become infected. Although *C. sepedonicum* survives poorly in soil (5), it could persist at low levels under some conditions not detectable with current research methods. Similarly, the possibility that certain native plants can either support soil populations of *C. sepedonicum* or serve as hosts has not been excluded. Spread among potato crops by insects, birds, or mammals may also be possible. Unfortunately, effective selective or enrichment media that would assist in detecting the bacterium from these sources have not been developed.

Recent Research Efforts and Future Goals

In recent years, research has focused on developing improved techniques for detecting *C. sepedonicum*. The development of a useful selective medium has proved frustrating. Hence, research efforts have emphasized methods for the direct detection of bacterial cells or their products. Because serological techniques fulfill the criteria of sensitivity and specificity, these have been explored most extensively. Various serological procedures, including latex agglutination (12), immunofluorescence (4,11), and immunodiffusion (3), have been studied for detecting the BRR bacterium. Each of these procedures has specific advantages as a laboratory test to confirm tentative BRR diagnoses made on the basis of plant or tuber symptoms (8). Although antisera with good specificity for *C. sepedonicum* have been produced, caution must be observed in interpretation of serological tests because cross-reactions with other bacteria have been demonstrated (2).

Sensitive assays, such as the immunofluorescence procedure, enhance detection of cross-reactions because antigenic sites in low frequencies on individual cells can be observed. Thus, in preparations with only a few *C. sepedonicum* cells, positive reactions with the pathogen cannot always be distinguished from reactions with other bacteria. One strategy for circumventing this problem has been to use the recently developed hybridoma technology to develop monoclonal antibodies, ie, a population of antibodies that all recognize the same antigenic determinant. If an antigenic determinant unique to *C. sepedonicum* exists, it may be possible to produce antibodies specific for that determinant. In preliminary work, monoclonal antibodies highly specific for *C. sepedonicum* have been produced (S. H. De Boer and A. Wieczorek, *unpublished*).

Considerable work needs to be directed toward understanding the parameters that affect disease development. Recent

work suggests that inoculum level, potato cultivar, and temperature are among the important variables (9; S. H. De Boer, *unpublished*; A. L. Bishop and S. A. Slack, *unpublished*). These parameters need to be quantified so their effect(s) on disease development can be predicted. Can we eventually predict whether symptoms should be expressed by a certain potato cultivar under a given set of environmental conditions? Indeed, what regulates development of the host-parasite relationship that leads to macroscopic disease symptoms?

Additional work on the ecology of *C. sepedonicum* is required. Do we really know all the inoculum sources for BRR? Can native plants or even potatoes support soil populations of *C. sepedonicum*? A selective enrichment medium would be extremely useful in such studies. Meaningful results will probably require selecting strains of the bacterium that can be uniquely identified (eg, antibiotic resistance genetic markers).

Summary and Recommendation

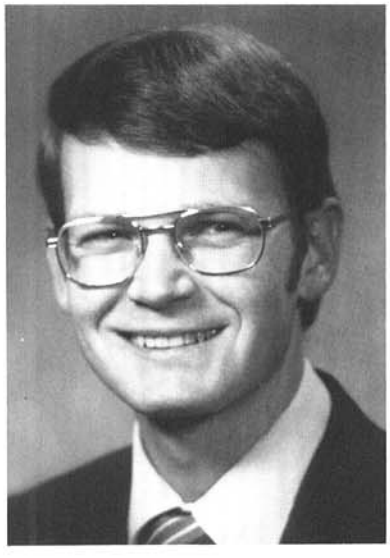
We must admit that our knowledge of BRR of potato has advanced only slightly during the last 50 years. Our understanding of the ecology of *C. sepedonicum* is still rudimentary. Efforts to understand how *C. sepedonicum* persists in nature and interacts with its host would undoubtedly strengthen our approach to disease control.

Recently, meristem-tip culture coupled with rapid multiplication procedures has been used to produce and maintain virus-free basic seed stocks and has provided great impetus for initial control of other seedborne diseases. Testing basic seed stocks for the presence of *C. sepedonicum* is highly recommended to assure that the initial stock used for seed propagation is free from the bacterium. Use of stocks produced and tested in this manner is increasing annually. Annual reintroduction of pathogen-tested stocks into seed potato increase programs requiring stocks to be renewed within a limited number of crop generations should have the effect of diluting BRR inoculum levels over time.

Control, ie, eradication, of BRR is a problem for the *entire* potato industry. As long as BRR is considered only a seed production problem, further headway on control will be difficult. Everyone should be concerned about sanitation and crop quality and health. Pathogen-tested stocks can be introduced into seed increase programs and contaminated stocks can be identified and rejected for certification. However, poor sanitation on a farm, at a processing plant, or in a vehicle that transports potatoes may provide the weak link that keeps *C. sepedonicum* cycling through the industry. A concerned and responsible industry working together could make a significant contribution toward reducing the incidence of BRR in North America.

Solke H. De Boer

Dr. De Boer is a research scientist with Agriculture Canada at the Vancouver Research Station. He earned a B.Sc. and M.Sc. in plant science at the University of British Columbia and, in 1976, a Ph.D. in plant pathology at the University of Wisconsin-Madison. He is working on bacterial diseases of potato and is especially interested in application of serological procedures in disease diagnosis and ecological studies of phytopathogenic bacteria.



Steven A. Slack

Dr. Slack is an associate professor of plant pathology at the University of Wisconsin-Madison. He received his B.S. and M.S. degrees in plant pathology from the University of Arkansas at Fayetteville and his Ph.D. in plant pathology in 1974 from the University of California at Davis. He completed a sabbatical study leave at Cornell University in New York in 1983. Dr. Slack is responsible for the Seed Potato Certification Program in Wisconsin, and his research has concentrated on the development and evaluation of techniques for the detection of potato pathogens in seed stocks.

Literature Cited

1. Bonde, R. 1939. Bacterial wilt and soft rot of the potato in Maine. Maine Agric. Exp. Stn. Bull. 396.
2. Crowley, C. F., and De Boer, S. H. 1982. Non-pathogenic bacteria associated with potato stems cross-react with *Corynebacterium sepedonicum* antisera in immunofluorescence. Am. Potato J. 59:1-8.
3. De Boer, S. H. 1983. Evaluation of an agar immunodiffusion procedure for confirming bacterial ring rot diagnoses. Am. Potato J. 60:661-669.
4. De Boer, S. H., and Copeman, R. J. 1980. Bacterial ring rot testing with the indirect fluorescent antibody staining procedure. Am. Potato J. 57:457-465.
5. Dykstra, T. P. 1942. Compilation of results in control of potato ring rot in 1941. Am. Potato J. 19:175-196.
6. Glick, D. P., Ark, P. A., and Racicot, H. N. 1944. Outline of procedure for the diagnosis of bacterial ring rot of potatoes; report of the Potato Association of America. Am. Potato J. 21:311-314.
7. Kreutzer, W. A., Glick, D. P., and McLean, J. G. 1941. Bacterial ring rot of potato. Colo. Exp. Stn. Press Bull. 94. 12 pp.
8. Manzer, F. E., and Slack, S. A. 1979. Report of the pathology section committee on bacterial ring rot diagnosis. Am. Potato J. 56:551-555.
9. Nelson, G. A. 1982. *Corynebacterium sepedonicum* in potato: Effect of inoculum concentration on ring rot symptoms and latent infection. Can. J. Plant Pathol. 4:129-133.
10. Shepard, J. F., and Claflin, L. E. 1975. Critical analyses of the principles of seed potato certification. Annu. Rev. Phytopathol. 13:271-293.
11. Slack, S. A., Kelman, A., and Perry, J. B. 1979. Comparison of three serodiagnostic assays for detection of *Corynebacterium sepedonicum*. Phytopathology 69:186-189.
12. Slack, S. A., Sanford, H. A., and Manzer, F. E. 1979. The latex agglutination test as a rapid serological assay for *Corynebacterium sepedonicum*. Am. Potato J. 56:441-446.
13. Starr, G. H., and Riedl, W. A. 1941. Bacterial ring-rot of potatoes. Univ. Wyo. Agric. Exp. Stn. Bull. 244. 12 pp.
14. Vidaver, A. K., and Starr, M. P. 1981. Phytopathogenic coryneform and related bacteria. Pages 1879-1887 in: The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel, eds. Springer-Verlag, Berlin.