POTATO RING ROT
IMPROVED METHODS FOR DETECTION AND DIAGNOSIS

by
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Importance of Ring Rot

Bacterial ring rot has a unique status among the diseases of potato. It is still the only disease for which all potato seed certification agencies will reject a seed lot on the basis of a single infected plant in a seed field or a single tuber in a seed lot shipment. This rule of zero tolerance became necessary because of the rapidity with which widespread infection resulted from a seed lot in which only a trace of infection was evident the previous year (Anon. 1957). These regulations, plus careful attention to sanitation and cultural practices, have made it possible to keep losses from ring rot to very low levels. Notwithstanding the overall beneficial results of such rigorous programs, severe losses still occur on individual farms. It must also be recognized that of the total seed acreage in the United States and Canada that is rejected annually for certification, approximately 60% is attributable to ring rot; this represents a significant added cost in the production of seed potatoes (Shepard and Claflin, 1975).

When ring rot is detected in a field the economic impact on a seed producer is obviously considerable. Thus, if ever there is a need for accuracy in disease diagnosis it is in the case of ring rot disease. Field diagnosis is based on foliar or tuber symptoms and it is usually confirmed by a Gram stain. In the hands of well-trained competent individuals one can have little question as to the validity of the diagnosis. However, one of the confusing aspects of ring rot is its sporadic appearance. The disease will sometimes appear without any apparent recent source of inoculum. In some cases the crop on certain farms becomes infected at intervals of three to four years. In other instances diseased plants are found once on a farm and may never be seen again after sanitation and other control measures are initiated. In many cases recurring problems can be traced to inadequate sanitation or cultural practices; however, many cases still occur for which no apparent reasonable explanation can be found.

Ring Rot Symptoms

Distinctive symptoms of ring rot often do not appear until growth of plants is well advanced. Furthermore, relatively warm temperatures (above 75° F) are essential for symptom expression. Thus, it is possible for plants and tubers to be infected but without any visible evidence that the causal bacterium may be present. It is this aspect of the ring rot disease that frustrates detection efforts during even the most meticulous inspection of seed fields.

Sometimes only one stem in a hill will show symptoms of wilting or stunting; other stems in the same hill may appear to be perfectly healthy. The leaves of infected stems change from their normal color to pale green; then areas between the veins become pale yellow and the leaf margins may roll upward. Plants wilt progressively and finally reach a state of irreversible wilting. The bacterium moves up the stem in the water-conducting tissue and through the stolon into the vascular system of developing tubers. The invaded tissues gradually turn yellow to brown as the disease advances. The bacteria multiply rapidly in the vascular ring and a milky white exudate containing millions of bacterial cells can be squeezed from the cut end of an infected stem. As the season progresses the ring rot organism spreads more rapidly in the root system of affected plants than above ground in the stems and the collapse of potato plants late in their growth period is mainly attributable to destructive effects of the parasite on the roots which eventually cease to function in the uptake of water and nutrients. When infected tubers are cut in cross-section, the vascular tissue may show a typical

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soft, creamy yellow or light brown discoloration and droplets of bacterial slime will be extruded from the discolored areas when pressure is exerted on the tuber. The skin of severely affected tubers will often crack open in an irregular fashion. At this point, secondary decay organisms become involved and the entire tuber decays.

Diagnosis and detection of ring rot is not a problem if the above typical symptoms of disease are present and good specimens are available for a Gram stain test. If seed tubers show no symptoms and produce plants without symptoms and daughter tubers also show no symptoms, highly sensitive detection methods are essential if in fact very low populations of the parasite are present. Furthermore, these improved techniques are needed for basis studies on the ecology of the ring rot bacterium. In addition to seed transmission, it is important to make a careful re-evaluation of the other possible sources of inoculum that make elimination of the ring rot bacterium so difficult.

The objectives of this paper are to outline some of the problems associated with the use of the Gram stain and to describe some of the new techniques involving serological detection that have been developed recently.

The Gram Stain

The most widely used differential stain of bacteria is the Gram stain first developed by the Danish bacteriologist, Christian Gram. On the basis of their reaction to the Gram stain, bacteria can be divided into two main groups: Gram-positive and Gram-negative. In general, most bacteria that cause disease of plants are Gram-negative. A small number of plant parasitic bacteria, including the organism that causes the ring rot disease, are Gram-positive bacteria, classified in the genus Corynebacterium.

The Gram-stain reveals fundamental differences in the cell wall structure of different bacteria. It might be well to briefly outline the specific process involved. Bacterial cells are smeared on a slide, heat-dried, stained with a solution of the dye, crystal violet, and then washed with water to remove excess dye. At this state all bacteria, both Gram-positive and Gram-negative, will be stained purple. The slide is then treated with a solution of iodine-potassium iodide. This iodine-potassium iodide enters bacterial cells and forms a water-insoluble complex with the crystal violet. Again both the Gram-positive and Gram-negative cells are affected to the same degree. A decolorization of the cells is then completed with ethyl alcohol. After decolorization Gram-positive cells remain purple, but the Gram-negative cells are colorless. In order to see the Gram-negative cells, a counter-stain, a red dye, such as safranin is applied. Thus, after the counter-stain the Gram-negative cells are red and the Gram-positive cells still remain purple.

Gram-positive and Gram-negative cells differ considerably in the structure of their cell walls. A Gram-negative cell wall is a multi-layered structure and very complex, whereas the cell wall of a Gram-positive organism, such as the ring rot bacterium, consists of a large single thick layer. It is because of this difference in cell wall structure that the insoluble complex formed between the potassium iodine and the crystal violet is not removed in decolorization with ethyl alcohol. It should be pointed out that Gram "positivity" is not an all or nothing phenomenon. Some organisms are more Gram-positive than others and some are Gram-variable being Gram-positive or Gram-negative depending on growth conditions and age of bacterial cells. Thus, although the Gram stain is one of the most common and reliable staining procedures in a bacteriological laboratory, it is also one which has to be used with some degree of interpretation and which lacks specificity. It cannot be used alone as the sole basis for a positive diagnosis of bacterial ring rot or any other plant disease incited by bacteria.

In some seed programs, it has been the practice to discard any plants from which smears are obtained that contain Gram-positive bacteria. This has been based on the assumption that the ring rot bacteria may be present and not produce symptoms and only appear

when those conditions are favorable for more rapid development and infection by the pathogen. However, a number of bacteria that will stain positively with the Gram stain may be present even in healthy plants (De Boer and Copeman, 1974). Gram-positive bacteria are frequently found that are distinguishable from the ring rot bacteria only by a few physiological characteristics and their inability to cause disease. Furthermore, it has been demonstrated that the stem smear method may not detect low populations of the ring rot bacteria as may be the case when conditions are not favorable for disease development.

Serological Procedures

A wide range of methods which were developed for the diagnosis of animal and human pathogens are now available for application in diagnosis of bacterial plant pathogens. In order to discuss these techniques, it will be necessary to outline very briefly certain basic serological concepts which are essential in understanding the procedures to be described.

All bacteria contain compounds known as antigens which are capable of inducing the formation of proteins called antibodies when they are introduced into the blood stream of man and other warm-blooded animals. Antibodies are detectable in the blood serum which is the liquid remaining after red blood cells are removed from a test animal into which bacteria has been injected at periodic intervals for several weeks. Each antibody recognizes and attaches to specific antigenic site(s) on the bacteria that stimulated antibody production. The reaction between antigen and antibody is extremely sensitive and remarkably specific. It is this specificity that is the basis for serological procedures.

When bacteria are suspended in a solution containing a low concentration of salt, they may remain in suspension for a relatively long time. However, if they are mixed with the antiserum containing antibodies specific to the bacteria involved, a rapid reaction occurs in which the bacteria clump and the reaction known as agglutination occurs. Then, it is possible to see visible aggregates of large numbers of cells and determine whether or not the antibody is present which was specific to the antigenic compounds present on that bacterium. It is important to recognize that the bacterial cell is not one homogeneous antigen but it is composed of many different antigens forming an antigenic complex. The antigens of the bacterial cells are distributed in the cell and cell wall as well as in the whip-like flagella which propel bacteria in water. Each species or strain of bacteria has its own set of antigenic complexes. The serum produced against one species of bacterium may have no antibodies against another one. However, two related bacteria may contain similar antigens. Thus, the crude serum produced against one species of bacterium may react with one or more antigens of another one and there are many instances in which common antigens are present in both.

The double diffusion test is based on the precipitation reaction which is completed in an agar layer in a small plastic dish called a petri dish. Bands of precipitate will form in the agar between tiny wells in which the antiserum and antigen are placed. By comparing the number and pattern of the bands one can draw conclusions about the antigenic structure and relationship of different bacterial strains. Antigenic structure of two bacteria can be said to be identical only if the two antigens, when placed in adjacent wells, form a fused precipitin band in tests with the antiserum for one of the strains.

Serological studies on the ring rot bacterium utilizing serological procedures have been reported previously by a number of investigators (Strobel and Rai, 1968, Claflin and Shepard, 1977). Recent studies at Wisconsin were initiated to evaluate the sensitivity and specificity of the complete range of serological techniques and determine how reliable these tests were on infected plant parts. Positive reactions were obtained in agglutination, double diffusion and in immunofluorescent antibody stain tests with known cultures of Corynebacterium sepedonicum obtained from Canada and the United States (Slack et al., 1978, 1979). No significant differences were noted among these strains in their relative sensitivity to the serological reactions involved. In contrast, seven other species or varieties of Corynebacterium pathogenic to plants did not react positively in double diffusion or immunofluorescent

antibody stain tests. However, a few cross reactions were obtained in agglutination tests. Unless some care is exercised in the agglutination procedure this technique may not always provide the degree of specificity that can be obtained with the other procedures. A number of Gram-positive bacteria, such as Bacillus spp., and anaerobic bacteria, such as strains of Clostridium, also did not react with the antiserum developed for the ring rot bacterium. No positive reactions were obtained with different species of the soft rot bacteria (Erwinia spp.) or Pseudomonas solanacearum, the causal agent of bacterial wilt or brown rot of potato. In these tests, the immunofluorescent antibody stain procedures were more sensitive than either the agglutination or double-diffusion tests. This higher level of sensitivity may not be essential to confirm diagnostic symptoms because the agglutination and double diffusion tests appear to be sensitive enough to detect the bacterium in visibly affected plant parts. The sensitivity was not appreciably affected by adding a culture of Bacillus polymyxa to reaction mixtures or by using potato tuber extracts rather than other suspending media.

The direct fluorescent antibody stain procedure was not used because the fluorescence lacked the desired intensity. It is evident from these recent investigations that serological testing for the ring rot bacterium can be used with a high degree of confidence to complement the current diagnostic criteria which are highly dependent upon the experience and skill of the individual diagnostician. It should be stressed, however, that serological tests, like other tests, must be performed correctly in order to obtain a reliable result that can be used as a diagnostic aid by a diagnostician.

Literature Cited

Anon. 1957. How can we interpret the zero tolerance and bacterial ring rot in certified seed potatoes. Report of the Seed Certification Committee of the Potato Association of America. Am. Pot. J. 34:142-148.

Claflin, L. E. and J. F. Shepard. 1977. An agglutination test for sero-diagnosis of Corynebacterium sepedonicum. Am. Pot. J. 54:331-338.

DeBoer, S. H. and R. J. Copeman. 1974. Endophytic bacterial flora in Solanum tuberosum and its significance in bacterial ring rot diagnosis. Can. J. Plant Sci. 54:115-122.

Shepard, J. F. and L. E. Claflin. 1975. Critical analyses of the principles of seed certification. Ann. Rev. Phytopathol. 13:271-293.

Slack, S. A., A. Kelman, and J. B. Perry. 1978. Evaluation of agglutination, Ouchterlony double diffusion, and indirect fluorescent antibody stain techniques for serodiagnosis of Corynebacterium sepedonicum. Amer. Pot. J. 55:395. (Abstr.)

Slack, S. A., A. Kelman, and J. B. Perry. 1979. Comparison of three serodiagnostic assays for the detection of Corynebacterium sepedonicum. Phytopathology 69: (In press).

Strobel, G. A. and P. V. Rai. 1968. A rapid serodiagnostic test for potato ring rot. Plant Dis. Reptr. 52:502-504.