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Detection of Seedborne Bacterial Plant Pathogens

Seeds have played and will continue to play a vital role in the development of modern societies. Without quality seed, a society's most important factor in survival, that is, production of food, will suffer.

Seeds frequently are transported from one country to another for breeding purposes and for direct production. In Europe, federal seed laboratories were established prior to 1900 to prevent seed adulteration and poor seed germination. The first official seed laboratory was established in Germany in 1869. Seven years later, such a laboratory was established in the United States by the Connecticut Experiment Station. Today most countries have numerous state and federal seed laboratories testing for purity, germination, noxious weed seeds, moisture content, and several seedborne fungi.

Seed testing stations are more numerous in Europe than in the United States and are more involved in assaying for seedborne pathogens. But even in Europe, testing seeds for plant-pathogenic bacteria has received little attention as a means of controlling seedborne bacterial pathogens. Much of this reluctance of seed testing stations to include assays for seedborne bacteria is due to the lack of proven assay techniques. Another reason has been the accepted concept among most pathologists that chemical seed treatments and/or field sprays can effectively control seedborne diseases. Chemicals have controlled some fungal diseases but have met with only limited success with bacterial diseases. A further hindrance to the development of seed assays is the misunderstanding that seedborne diseases can be reliably detected by field inspections. Thus, few successful methods of assaying seeds for plant-pathogenic bacteria are available.

Because of the increased demand for seed needed for world food production and the availability of rapid air transportation, several countries require phytosanitary labels for certain imported seeds known to carry seedborne bacteria. This increases the pressure on seed producers to offer seeds that are free from certain pathogens. Field inspections have been required in the past for several certified seed programs and have helped eliminate many highly infected seed lots but certainly not lots with low levels of infection. In fact, in Michigan 25% of all field-certified bean seed lots contained infected seeds (3).

With the increased use of sprinkler irrigation in seed crops and the availability of laboratory assays for certain pathogens, seed purchasers are beginning to demand evidence that the purchased seed lot was assayed and shown to be free from the pathogen. Requests also are being made for assays of treated seed lots. Ralph (10), discussing problems in testing and control of seedborne bacteria, concluded that ". . . despite many complicating factors, growing-on tests carried out over a number of years and/or under varying environmental conditions provide the most reliable means of assaying seed treatment chemicals." An article on testing seeds for seedborne organisms in the 1961 USDA Yearbook of Agriculture discusses only two methods, growing-on and phage-plaque count tests, for seedborne bacteria (1).

It is not possible to determine if a seed lot is free from infected or infested seeds but it is possible to certify that a seed lot contains less than a specified level of infection. This can best be determined by seed assays. Field inspections are of value in certification schemes, but some caution should be taken. Certified bean seed is grown in the western United States because secondary spread of bacterial diseases usually does not occur in furrow-irrigated crops in climates with less than 1 in. of rainfall during the growing season (8: Mackie et al 1945). Wilson, however, reported that it was difficult to detect trace infections in plants grown under low rainfall (23). He suggested that clean seed could be obtained more reliably by sowing seed shown to be free from halo blight organism in an area with sufficient rainfall to favor spread and facilitate detection of the pathogen, if present. In fact, bean seeds can be infested with *Pseudomonas syringae* pv. *phaseolicola* (*P. phaseolicola*) under low rainfall conditions in the Central Valley of California without symptoms being evident in the field (8: Grogan and Kimble 1967). Grogan and Kimble recommended that infested stock seed should not be used for certified seed production.

Other factors also reduce the usefulness of field inspections. For example, resistant bean cultivars may show no symptoms of common blight, yet the pathogen, *Xanthomonas campestris* pv. *phaseoli* (*X. phaseoli*), can be seed-transmitted (2); seed assays are the most reliable method of determining if the seeds are infected with *X. phaseoli* (2). Symptoms of seedborne bacterial diseases can be masked by other diseases. Downy mildew of crucifers makes it very difficult to observe symptoms of black rot. We also have observed that symptoms are better expressed in young vegetative plants than in flowering plants of cabbage (G. V. Minsavage and N. W. Schaad, *unpublished*). *X. campestris* pv. *campestris* (*X. campestris*) is a high-temperature organism growing best at 30 C, whereas crucifers are cool-season plants. Optimum growth conditions for the host, therefore, are not optimum for development of black rot symptoms. We know that symptoms can be masked by holding plants at 15–20 C but quickly become obvious when the temperature rises to 25–30 C (N. W. Schaad, *unpublished*). Since temperatures are generally low during the vegetative growth stage of crucifer seed plants, one would not always expect diseased plants to be easily detected.

Crucifer seeds are produced in two major areas in the United States, central coastal California and western Washington. Black rot has been observed occasionally in seed fields in California but never in Washington. In 1980 a field survey showed that black rot was common in cruciferous weeds and commercial crucifer seed plants in California but was not observed in Washington (N.W. Schaad, *unpublished*). *X. campestris* was isolated, however, from three seed lots grown in Washington in 1980 (N. W. Schaad, *unpublished*). We cannot be certain if the failure to observe black rot in plants in Washington is due

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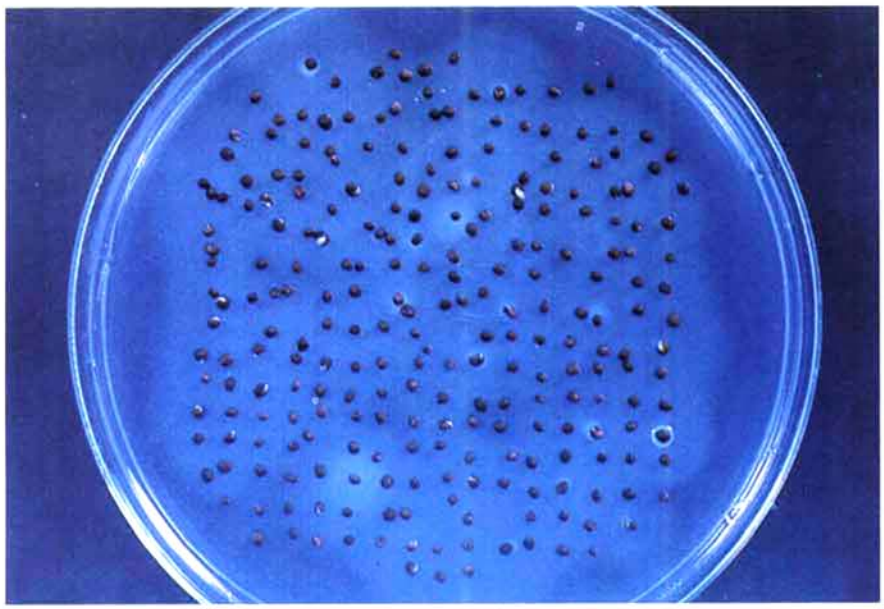

Fig. 1. Direct plating of surface-disinfested crucifer seeds onto SX agar, a semiselective medium for *Xanthomonas campestris*, using a vacuum seed spotter. Note the single seed surrounded by a colony of *X. campestris*.

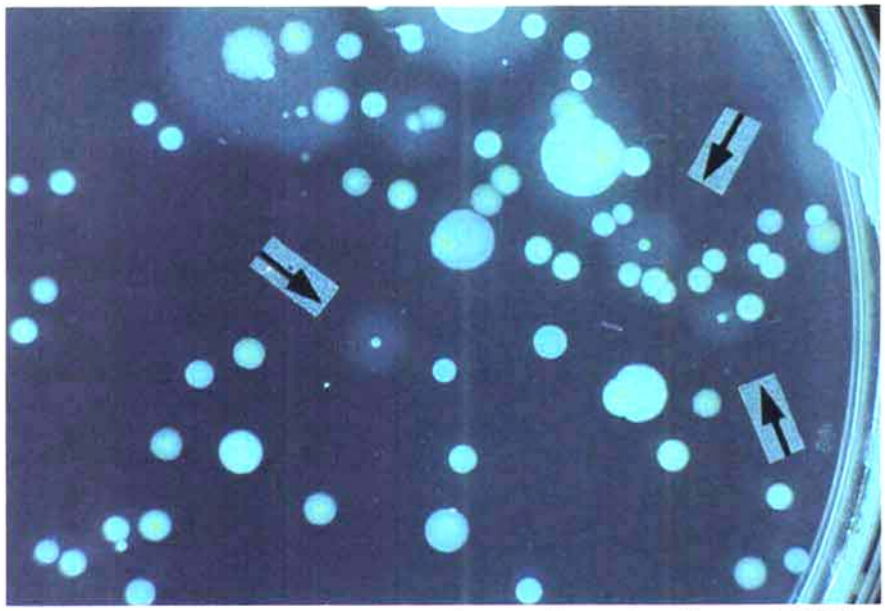

Fig. 2. Isolation of *Xanthomonas campestris* from crucifer seed washings onto NSCA, a nutrient, starch, and cycloheximide agar. Small yellow colonies surrounded by a zone of starch hydrolysis (arrows) are *X. campestris*.

to a masking of symptoms by low temperatures or by other complicating factors such as fungal diseases, but we are confident that seed assays are a more reliable method of determining the presence of *X. campestris* in such seed lots.

Recently there has been considerable interest in developing more reliable and sensitive assays for detecting seedborne bacteria. Under the leadership of the Bacteriology Working Group of the Plant Disease Committee (PDC) of the International Seed Testing Association (ISTA), formed in 1975, comparative testing of assay methods has become a reality on an international scale. The committee gives the highest priority to comparative seed health testing. Both the American Phytopathological Society (APS) and the International Society for Plant Pathology (ISPP) now have seed pathology committees, and regional Cooperative States Research Service committee NC-135 devotes itself entirely to seedborne bacteria. Despite this interest in seedborne bacteria, no service within the USDA exists for testing seedborne bacteria. With over 45 plant-pathogenic bacteria known to be seed-transmitted (7), it is no wonder that bacterial diseases are of major economic importance.

Further complicating the control of seedborne bacteria is the general practice of treating seeds with chemicals or hot water to eliminate seedborne fungi and/or bacteria without assaying the seeds to determine if such treatment is needed or successful. This is especially true with the hot water treatment, a standard recommendation for crucifer seeds. In fact, hot water treatments are not always successful. Srinivasan et al (8: 1973) reported finding *X. campestris* in several seed lots that had been commercially treated with hot water. We found experimentally that hot water treatments did not always eradicate *X. campestris*. Furthermore, in 1981, a seed lot that tested positive for *X. campestris* in our Georgia State Department of Agriculture seed health assay program was resubmitted by the seed company after being treated with hot water; the seeds were assayed and *X. campestris* was isolated. Thyr (8: 1969) reported that hot-water-treated tomato seeds caused problems in agar plating assays for *Corynebacterium michiganense* by increasing the numbers of contaminating fungi. It is clear that treated seeds can complicate seed assays and that treatments cannot always be assumed to have been successful. It is important, therefore, that seed assay samples be submitted before treatment.

Seed assay methods have been developed for many seedborne bacteria and with slight modifications might easily be adapted to other bacteria. Such methods as growing-on, plant injection, bacteriophage multiplication, direct isolation on agar media, and serology have been used to assay for seedborne bacteria (Table 1). Several of these methods are being used for state seed certification schemes (3,8: Ednie and Needham 1973,12,18), but none has been tested thoroughly enough to be recommended by ISTA.

Developing a Seed Assay

Growing-on tests have been used more than any other method to detect seedborne bacteria. Initially, seeds were simply sown in the field or greenhouse and the development of the disease recorded. Variations include using 8-in. pots (8: Grogan and Kimble 1967), special plastic trays (9), and "cake domes" (J. R. Venette, *personal communication*) as humidity chambers to allow optimum conditions for the bacterium to infect the seedlings. Another form of growing-on test is performed in the laboratory. Seeds are placed on wet paper towels (8: Shackleton 1962) or on water agar in petri dishes (8: Srinivasan et al 1973) and the resulting cotyledons are observed under a dissecting microscope for symptoms. Knowledge about infectivity of the causal organism can be helpful. For example, wounding seeds by shaking them in sand before sowing in vermiculite increased detection of *P. syringae* pv. *glycinea* (9).

Although growing-on tests offer the advantage of determining seed transmission, they are not well suited to testing numerous commercial seed lots. The tests are time-consuming, and a considerable amount of greenhouse or growth chamber space is required for testing 10,000 or 50,000 seeds per seed lot. They also are complicated by seedborne fungi that can confuse symptom expression and/or kill the seedlings. Seedborne fungi are a problem with certain crucifer seed lots. Furthermore, an infected seed may fail to germinate and therefore go undetected. Such seeds could provide inoculum for root infection of adjacent plants under field conditions. Finally, infection may not occur if proper environmental conditions are not maintained.

Laboratory assays have the decided advantages of being faster, easier to perform, less expensive, and more sensitive. Development of a successful laboratory assay can be divided into three major steps: 1) extraction of the bacterium from the seed, 2) identification of the extracted bacterium, and 3) determination of assay sensitivity and tolerance levels.

Extracting the Bacterium

Successful recovery of the pathogen from seed requires an understanding of the relationship of the pathogen to the seed and to other microflora. Is the bacterium present in dust on the surface or in cracks, as with *P. phaseolicola* (8: Grogan and Kimble 1967), or is it internal in the funiculus of the seed, as with *X. campestris*? Several methods have been used to recover bacteria from seeds. These methods usually involve soaking seeds in liquid media for several hours (4,6,9,18,19,22) or washing the seeds briefly in a liquid medium (12). Seeds can be surface-disinfested first (4,10,11,13,19) or left untreated (4,9,12,18,20,22). The seeds can be plated directly onto a partially selective medium (13), ground dry in a hammer mill (8: Ednie and Needham 1973, Taylor 1970, Thyr 1969), or blended wet in a Stomacher (8: Taylor 1970) or comminuted in a blender (4,8: Katznelson 1930, Kennedy 1969).

In choosing a method, one should first run controlled experiments using several different sources of seed. One cannot expect each pathogen to be extracted efficiently by a single method or the microflora of one seed lot to be the same as another. For example, we find that *X. vesicatoria* is not easily washed from the surface of tomato seeds (N. W. Schaad and D. Pinnow, *unpublished*). Cabbage seed received from different fields in Japan, California, or Washington have very different microflora. Soaking bean seeds from some lots in water or nutrient broth prevents subsequent isolation of *P. phaseolicola* because of contaminant bacteria (8: Taylor 1970). We observed similar results with *X. campestris* on crucifer seeds.

Because naturally infected seeds are difficult to obtain, preliminary experiments can be performed using seeds mixed with finely ground infected leaves (8: Grogan and Kimble 1962), soaked in a pure culture of the bacterium, or inoculated by a pinprick-vacuum technique (13). Eventually, however, naturally infested seeds should be used. Determining what method of extraction is best may require considerable effort but is very important. For example, we found with certain seed lots that the number of viable cells of *X. campestris* was often reduced when seeds were ground. In some cases, *X. campestris* could not be isolated from known naturally infested seed lots. We also found that when seeds were incubated in water, the number of recoverable viable cells of *X. campestris* always decreased. Further checking revealed that crucifer seeds often contained bacteria antagonistic to *X. campestris*. Among eight seed lots tested, antagonistic bacteria were found on 0.5–12% of the seeds (N. W. Schaad and R. C. Donaldson, *unpublished*).

A further complicating factor in attempting to grind or incubate crucifer seeds is the possibility that the seeds contain chemicals inhibitory to *X. campestris*. Incubating bean seeds to increase the numbers of *P. phaseolicola* was not successful because rapid multiplication of saprophytic bacteria prevented recovery of *P. phaseolicola* (8: Taylor 1970). If an enrichment technique still seems preferable, one could identify the saprophytes and/or antagonists and determine their sensitivity to various antibiotics and/or chemicals. The inhibitory compound(s) could then be added to a liquid medium to selectively increase the growth and ultimate recovery of the pathogen. Such a medium is used for detecting *X. phaseoli* in beans (19).

The most reliable and practical method for recovering *X. campestris* is to wash nondisinfested seeds in saline plus a detergent such as Tween 20 (12). Direct plating of surface-disinfested seeds onto SX agar, a selective medium containing starch and dyes (13), is efficient but very time-consuming (Fig. 1). An advantage of the surface-washing technique is that bacteria in the washing can be concentrated and several different methods, such as agar plating, serology, or phage, can be used to identify the pathogen. With *P. phaseolicola* on bean, Taylor (8: 1970) recommends grinding seeds in a hammer mill or blending seed in a Stomacher, whereas Trigalet and Bidaud (18) recommend soaking seeds for 6–10 hours in sterile water.

Table 1. Assay methods used for detecting seedborne plant-pathogenic bacteria

Assay method	Species or pathovar	Host	Reference
Growing-on	*Pseudomonas glycinea*	Soybean	9
	P. phaseolicola	Bean	8:Grogan and Kimble
	P. pisi	Pea	8:Watson and Dye
	Xanthomonas campestris	Crucifers	8:Shakleton; Srinivasan et al
	X. carotae	Carrot	8:Ark and Gardner
	X. incanae	Stock	8:Kendrick and Baker
	X. nigromaculans	Zinnia	17
	X. oryzae	Rice	16
	X. phaseoli	Bean	8:Schuster and Coyne
	X. vesicatoria	Pepper	15
Plant injection or inoculation	*Corynebacterium michiganense*	Tomato	8:Thyr
	P. glycinea	Soybean	8:Kennedy
	P. phaseolicola	Bean	8:Wharton
	X. phaseoli	Bean	8:Saettler
Seed culturing	*X. campestris*	Crucifers	13
	X. nigromaculans	Zinnia	17
Direct isolation	*P. phaseolicola*	Bean	8:Taylor, Wallen and Sutton
	X. campestris	Crucifers	6,12,13
	X. phaseoli	Bean	8:Ednie and Needham; 19
Phage	*C. michiganense*	Tomato	8:Ercolani
	P. atrofaciens	Cereals	8:Sutton
	P. phaseolicola	Bean	8:Taylor, Sutton and Katznelson
	P. pisi	Pea	8:Sutton and Katznelson
	X. oryzae	Rice	21
	X. phaseoli	Bean	4
Serology	*P. phaseolicola*	Bean	8:Coleno; Guthrie et al; 18
	X. campestris	Crucifers	12

Identifying the Bacterium

The method most commonly used to identify seedborne bacteria has been the injection of seed comminutions or purified preparations of the isolated and purified bacterium into susceptible host plants to observe symptoms. Characteristic symptom development provides direct evidence of the pathogen. The major disadvantage is the time required for symptom development and interpretation of results. The interpretation of results is especially a problem with pseudomonads. Symptom expression does not necessarily prove pathogenicity. Pseudomonads are widespread, and many will produce symptoms on nonhosts when plants are grown under adverse conditions or inoculum dosages are high (11). Furthermore, inoculation of beans with *P. phaseolicola* results in very few lesions when the temperature is 28 C or greater (11). It is apparent that one must use controlled conditions and be knowledgeable of symptom characteristics when testing for pathogenicity, especially with leaf-spotting pseudomonads.

One laboratory method used to identify bacteria directly in seed washings or comminutions is the phage-plaque multiplication method of Katznelson (4). The technique has been used to identify *X. phaseoli* and *P. phaseolicola* in bean seeds, *P. syringae* pv. *pisi* (*P. pisi*) in pea seeds, and *C. michiganense* in tomato seeds. Ednie and Needham (8: 1970) used phage to identify *X. phaseoli* pv. *fuscans* isolated onto agar media from comminuted beans. Phage also has been used to identify *P. phaseolicola* isolated from ground bean seeds (8: Taylor 1970).

Phage is a simple and rapid method of identification, but extensive tests must be run to determine specificity. A major disadvantage of the direct phage-plaque method of identifying the bacterium in seed comminutions or washings is the lowered sensitivity when large numbers of other bacteria are present (8: Ednie and Needham 1973). Perhaps the greatest disadvantages of using phage are the usual lack of a species-specific host range and the resistance of some strains of the bacterium. With *X. campestris*, 18–20% of the strains tested were resistant to *X. campestris* phage (5, J. W. Sheppard, *personal communication*). Still, phage tests for identification have proved successful in Canada, where phages Pg 60 and Pg 176 have been used for about 20 years to identify *X. phaseoli* isolated from beans (J. W. Sheppard, *personal communication*). The Canadian workers have observed that all strains with a positive phage reaction are pathogenic, so testing the pathogenicity of such strains is not necessary.

With proper use of selective and/or differential agar media, presumptive identification of the genus is simple and requires few, if any, biochemical tests. Specific agar media are used for presumptive identification of seedborne bacteria in seed washings and comminutions and offer several advantages over plant injection methods. Identification on agar media is inexpensive, relatively fast, and easy to perform and, perhaps of more importance, results in a culture of the suspected pathogen. The problem is that suitable media have not been developed for most seedborne bacteria. Although King's medium B is helpful for isolating pseudomonads such as *P. pisi* and *P. syringae* from pea seeds and *P. phaseolicola* from bean seeds (8: Taylor 1970, 18), one still has difficulty distinguishing pathogenic pseudomonads from saprophytes. *X. campestris* can be isolated and differentiated from most saprophytic bacteria on a beef peptone and starch agar (6) or NSCA, a nutrient, starch, and cycloheximide agar (13), because of its ability to hydrolyze starch (Fig. 2). J. Taylor (*personal communication*) prefers to use NA, a less rich medium, to reduce growth of saprophytic bacteria. The disadvantage of NA, however, is that colonies of *X. campestris* are difficult to distinguish from colonies of other yellow-pigmented bacteria.

SX agar is preferred over NSCA when seeds are heavily contaminated with saprophytic bacteria (N. W. Schaad, *unpublished*, and J. W. Sheppard, *personal communication*). The surface-washing technique (12) combined with SX agar is being tested in Canada for *X. campestris*. Preliminary results showed that 31 of 141 (22%) lots tested during 1980–1981 were positive for *X. campestris* based on phage identification (J. W. Sheppard, *personal communication*).

Final identity of bacteria presumptively identified on agar media does require confirmation by time-consuming pathogenicity tests. Time poses no problem for routine identification, but test results must be available quickly for seed certification programs. Perhaps the best method for final identification that is both rapid and specific is serology.

Serology has been used to identify seedborne bacteria directly in enrichment culture of bean seeds. Guthrie et al (8: 1965) incubated surface-disinfested bean seeds for 36 hours in water and tested the leachate by agglutination and/or agar double-diffusion using antiserum against cells of *P. phaseolicola*. A similar method was used to detect *P. phaseolicola*, except the bacterium was identified by immunofluorescence (8: Coleno 1968); this method is more sensitive and uses less antiserum but requires a more specific antiserum.

The main advantages of serological tests are speed and low cost. The serological test perhaps best adapted to identification of seedborne bacteria is immunofluorescence. As little as 10 μl of antiserum is needed for each sample, and results are available in a single day. Immunofluorescence is routinely used by medical diagnostic laboratories, such as the Center for Disease Control in Atlanta, to identify clinical specimens. A major disadvantage of serology is that the method for each organism must be thoroughly studied. The specificity and reliability of any serological method depend on the specificity of the antiserum. This is especially so with immunofluorescence. Whereas minor cross-reacting antibodies can be distinguished readily in agar diffusion plates, such qualitative differences are not detected by immunofluorescence. The time spent identifying a species-specific antigen will save considerable time later. A monoclonal antibody would be a great advantage for immunofluorescence.

Another serological method that may eventually prove useful is ELISA. Until technical problems of detecting small numbers of bacterial cells (rather than antibodies or simple structures such as viruses) are worked out, however, the technique will not be very helpful. ELISA might work quite well for detecting bacterial toxins or enzymes.

Once workable methods of detecting seedborne bacteria are available through public and/or private laboratories, feasible certification schemes for controlling seedborne bacteria will become a reality. Detection sensitivity and tolerance levels must, however, be established first.

Determining Tolerance Levels

Few plant bacteriologists have shown much interest, until recently, in determining quantitatively the role of seedborne inoculum in the development of field epidemics. In France, an extensive 6-year study using naturally infected commercial seeds has shown that five infected bean seeds per 10,000 result in epidemics of halo blight of bean, whereas one per 20,000 does not (18).

The relationship of amounts of seedborne *X. campestris*, as determined by agar plating assays, to the incidence of black rot in the field has been determined. Seeds infected naturally with *X. campestris* were mixed with healthy seeds, assayed, and seeded in a field in South Carolina. Laboratory assays were successful in detecting the pathogen at 0.01% level of infection. The incidence of black rot was high in field plots that initially contained plant infections of 0.03% or greater but not in plots with 0.01% (14).

Certification Schemes for Seedborne Bacteria

A formal control order for inspection and destruction of halo-blighted bean fields was established in Idaho in 1964. If halo blight is found, the grower must destroy the field by plowing within 5 days. Although halo blight symptoms are readily diagnosed in most cases, symptoms on some cultivars are easily

confused with physiological or mechanical causes (8: Guthrie et al 1975). Serological tests are used to identify the isolated bacterium instead of pathogenicity tests, which take 14–21 days.

Blight-free breeder seed obtained from the Idaho certification program is used in Canada for producing bacterial blight certification of white beans (J. W. Sheppard, *personal communication*). A zero tolerance, based on two field inspections and a laboratory assay (8: Ednie and Needham 1973) of 30,000 seeds, is required for the "Select" seed (first generation). Foundation seed is field-inspected once, but a positive laboratory seed test results in rejection for certified seed production. Certified seed fields are inspected once and if disease is greater than 1%, a sample of 10,000 seed is assayed. A positive seed test results in rejection of the seed. Rigid enforcement of the "Select" program has apparently reduced bacterial blight to a reasonable level in Canada.

A certification program in Michigan for common blight of beans permits 0.005% blighted plants during field inspection and no infected seeds in laboratory tests (3). A sample is taken, surface-disinfested, and soaked in water for 24 hours, and the leachate is injected into bean seedlings (8: Saettler 1971). Results in Michigan have clearly shown that seed assays are preferable to field inspections, as 25% of the seed lots that had passed field inspections had to be rejected when the seeds were assayed (3).

In Georgia, all crucifer seed lots sold for transplant production must be assayed for *X. campestris*. Since the tests were initiated in 1976, *X. campestris* has been detected in 60 of 1,082 (5.5%) seed lots submitted for assay. Of 423 lots tested in 1980 by agar plating and immunofluorescence, 21 (5%) were positive by both assays, 23 (5.4%) by agar plating only, and 50 (11.8%) by immunofluorescence only. Although field plots have established a zero tolerance in 10,000 seeds for the agar assay, no tolerance has yet been established for the immunofluorescence tests. In 1981, 100,000 seeds from each of six commercial seed lots that tested positive by immunofluorescence and negative by agar plating were sown in a field at Experiment, Georgia, and a field at Davis, California. Approximately 250,000 seeds of each lot were obtained. A 10,000-seed sample was withdrawn for testing to be sure the samples were representative of the original sample (ie, agar-plate negative, immunofluorescence-positive). Each sample of 100,000 seeds was divided into two subsamples and sown in separate plots. Black rot developed in four of 12 subplots (three of six seed lots) in Georgia and three of 12 subplots (two of six seed lots) in California (R. N. Campbell, *personal communication*). Samples of the two lots

field-positive in both Georgia and California were positive by agar plating in the second assay, however. Therefore, only one of six immunofluorescence-positive, agar-plate-negative seed lots was positive in the field. Apparently, many cells identified as *X. campestris* by immunofluorescence were nonviable, were unable to infect, or were not *X. campestris*. These results illustrate the disadvantage of a seed assay that does not result in recovery of the pathogen and the problems of sampling.

Research Needs

Great strides have been made recently in seed pathology in general and in assay techniques in particular. The establishment of seed pathology committees by ISPP and APS, the PDC Bacteriology Working Group of ISTA, and NC-135 will certainly contribute much toward advancing our knowledge of seedborne bacteria. For example, NC-135 and the PDC Bacteriology Working Group are cooperating on the preparation of a laboratory guide on assay methods for seedborne bacteria.

Work in the following seed health research areas is certainly needed:

1. Epidemiology of seedborne diseases: Epidemiological data are needed to determine when and how to obtain the seed sample used for assaying. Data also are needed on correlation between laboratory assays and disease development so that assay tolerance levels can be established.

2. Identification: a) Selective and differential media. Media for isolation and identification are needed for most seedborne bacteria. This is perhaps the greatest need in the development of a successful assay method. b) Serology. A more critical evaluation of the specificity of antisera is needed. Development of monospecific antisera is especially needed for use in immunofluorescence. Standardized methods need to be established and a central antisera bank made available. Further research on the specificity of ELISA is needed, also. c) Phage. Further research with phage susceptibility and specificity is needed.

3. Certification programs for stock and breeder seeds: A greater emphasis is needed on assaying stock and breeder seeds. Many field problems can be eliminated by making pathogen-free breeder seed available.

Acknowledgments

I wish to acknowledge A. W. Saettler, J. W. L. van Vuurde, A. Trigalet, and J. W. Sheppard for making suggestions and sharing unpublished information.

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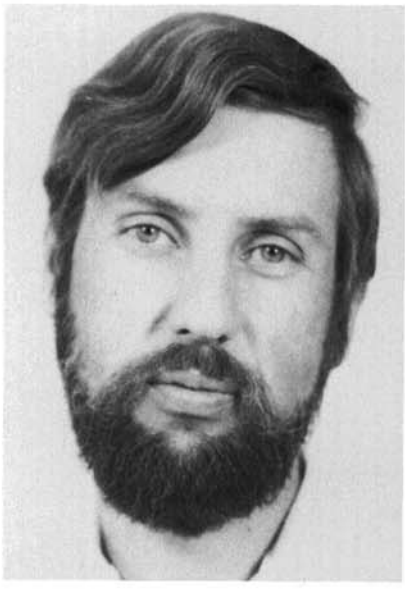

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