Inoculum Thresholds of Seedborne Pathogens

Bacteria

N. W. Schaad

Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow 83843.
Published as Idaho Agricultural Experiment Station Journal Series article no. 8776.
Accepted for publication 15 October 1987.

Many bacterial plant pathogens are seedborne (8). Such pathogens can result in heavy losses when disease is seed transmitted because infection occurs early and inoculum is evenly distributed throughout the field. Because bacteria can spread very quickly under suitable weather conditions, low levels of seedborne inoculum can result in severe epiphytotics. This is especially a problem with crops grown from transplants. A single infected plant (such as cabbage infected with *Xanthomonas campestris* pv. *campestris*) in a transplant bed could potentially provide enough inoculum to infect a high percentage of the plants. The problem can be complicated if symptoms do not develop before the transplants are shipped. Few bacterial diseases can be controlled in the field. Therefore, considerable effort has gone into the control of seed-transmitted diseases by using "pathogen-free" seeds. An important factor in a scheme of providing such seeds is the availability of a reliable seed assay to detect contaminated seeds.

WORLDWIDE IMPORTANCE OF SEEDBORNE BACTERIA

With increased use of hybrid seeds and the widespread distribution via air transportation, the need to control seedborne bacteria has intensified. Most seed is produced in one country and shipped by air to many other countries. Currently, 11 seedborne bacteria are regulated by five or more countries (Table 1). The European and Mediterranean Plant Protection Organization (EPPO) has defined two types of quarantine organisms. The seedborne pathogens in A-1 include those not present in the EPPO region, and those in A-2 include pathogens present but subject to international phytosanitary measures to prevent further spread. Two of four and seven of 18 organisms in A-1 and A-2, respectively, are bacteria (16). In contrast to Europe, little regulation of seedborne bacteria occurs within the United States. When regulations do occur, few are based on laboratory assays and field tolerances. Although resistance has been successful in controlling bacterial diseases, resistance is not always available. Other cultural controls, such as rotations, foliage sprays, seed treatments, or use of seed produced in semiarid climates, have been used with limited success. For example, much of the world's bean seed is produced in Idaho. Most of the U.S. bean seed industry moved to the irrigated desert of southern Idaho in the 1920s because of the dry climate. However, the climate in southern Idaho has apparently changed in the last 10 years due in part to the widespread use of sprinkler irrigation (H. Fenwick, *personal communication*). The climate is still much drier than that of the east coast but certainly not as dry as it was 60 years ago. Due to severe epiphytotics of bacterial blights in the early 1960s, Idaho has adopted very strict regulations, including laboratory tests and field trials (22). All seed to be grown in Idaho must pass laboratory assays. Seed grown for export is field-inspected several times each season and a zero tolerance is enforced. Infected fields are to be destroyed by plowing within five days. The system had helped improve the seed health quality of bean seed. However, because bacterial blights occasionally occur in Canada from seed lots from Idaho, the seed health of Idaho seed has been questioned recently (14). After a severe blight epidemic in Idaho in 1984, state investigations began, to develop an improved, rapid, sensitive method of assaying bean seeds for the two major bacterial blight pathogens in Idaho, *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola*.

SEED ASSAYS: THE CORNERSTONE TO CONTROLLING SEEDBORNE BACTERIA

Seed assays can provide information for 1) issuing seed health certificates, 2) need and effectiveness of a treatment to eradicate a specific pathogen, and 3) quarantine needs. For example, seed treatments that only reduce the level of inoculum may still be of great value if a zero tolerance is not needed for a particular pathogen. The contaminated seed could be treated and assayed to determine whether the level of contamination had been reduced below the tolerance level. The seed would therefore not have to be discarded.

Seeds are often treated to control seedborne organisms without researchers knowing whether the seeds are actually contaminated. In addition, the success of the treatment is seldom determined. For example, hot water is still considered to be the standard treatment for eradicating *X. c.* pv. *campestris* from crucifer seeds (1) and *X. c.* pv. *carotae* from carrot seeds (20) when in fact the treatments are not always successful (12, N. W. Schaad, *unpublished*). Similarly, organic mercuries were recommended for years for controlling black chaff of wheat (2,5). After mercury was banned as a seed treatment, many blamed the U.S. Environmental Protection Agency for the sudden increase in black chaff. No one questioned possible changes in the weather, introduction of newer, more

©1988 The American Phytopathological Society

susceptible cultivars, or increase in seedborne inoculum in breeder foundation seed stocks. The problem with many seed treatments is that the effectiveness of the treatment has often been determined only by sowing seeds in the field and observing symptoms. Such growing-on type assays are not very sensitive. Results of growing-on tests rely on proper environmental conditions for symptom development and can be confused by the presence of other pathogens. With black chaff, laboratory assays and field plantings of contaminated seeds treated with Gallotox and Ceresan MDB confirm that mercuries are ineffective (Forster and Schaad, *unpublished*). Had a laboratory assay for *X. c.* pv. *translucens* been available, mercury seed treatments probably would not have been recommended for controlling black chaff of wheat.

Because of increased costs of hybrid vegetable seeds, losses in transplant and field production and lawsuits, many growers are demanding that their seeds be assayed. For example, many crucifer transplant producers in California are requesting that seed companies assay their seed for *X. c.* pv. *campestris*. In Georgia, state regulations require that all crucifer seed purchased for transplant production be assayed for *X. c.* pv. *campestris*. This means that a reliable assay must be available. Responding to the increased demand by the seed trade and importing nations for improved methods for detecting seedborne bacteria, the Plant Disease Committee of the International Seed Testing Association (ISTA) established a Bacteriology Working Group in 1976. This group provides leadership in developing and evaluating methods for detecting seedborne bacteria.

To be effective, assays should be based on sound, epidemiological data (6). Studies of disease epidemiology are necessary to provide a practical guide to the levels of seed infection or contamination that are likely to result in serious disease outbreaks (i.e., to determine tolerance limits for seed contamination). Besides pertaining to field disease, assays should be reproducible, economical, and rapid.

Several types of assays for seedborne bacteria have been proposed and used, but results seldom have been related to field disease tolerances (Table 2).

Black chaff, caused by *X. c.* pv. *translucens*, is a serious disease in wheat and barley worldwide. The disease was first described by E. F. Smith in 1917 (15) and the pathogen was shown to be seedborne in barley seed from Montana in 1917 by L. R. Jones (5). Recently, the disease has become widespread in irrigated wheat in southern Idaho, where 90% of the state's soft white wheat is grown. This is important, because 80% of the wheat in southern Idaho is irrigated. Because *X. c.* pv. *translucens* is seedborne, we wanted to determine the extent and level of contamination in Idaho seed. However, no laboratory assay method was available. Because selective agar media have been very useful for seed assays (8), we first developed a semiselective medium (XTS agar) (11). In a survey of 60 commercial soft white wheat seed lots from southern Idaho in 1985, we found that 72% were contaminated (Schaad and Forster, *unpublished*). *X. c.* pv. *translucens* is not limited to Idaho seed, as we have isolated *X. c.* pv. *translucens* from seed from Montana, Washington, and Kansas. Three of 10 hard red winter wheat seed lots from Kansas were positive. In California, 38% of the seed lots assayed on XTS agar were positive for *X. c.* pv. *translucens* (Bob Webster, *personal communication*).

Although many seed lots in Idaho are contaminated, results comparing laboratory assays and field disease of two wheat cultivars in Idaho show that a zero tolerance is not necessary for control of *X. c.* pv. *translucens* (Table 3) (11).

TABLE 1. Seedborne bacteria regulated by five or more countries

Pathogen	Host	Regulating countries (no.)
Xanthomonas campestris		
pv. *campestris*	*Brassica*	7
pv. *vesicatoria*	*Capsicum*	8
pv. *phaseoli*	*Phaseolus*	10
pv. *phaseoli* var. *fuscans*	*Phaseolus*	7
pv. *stewartii*	*Zea*	14
Pseudomonas syringae		
pv. *lachrymans*	*Cucumus*	5
pv. *phaseolicola*	*Phaseolus*	10
pv. *pisi*	*Pisum*	>17
Clavibacter michiganense		
subsp. *michiganense*	*Lycopersicon*	>20
subsp. *insidiosum*	*Medicago*	16
Corynebacterium flaccumfaciens		
subsp. *flaccumfaciens*	*Phaseolus*	17

NEED FOR INFORMATION ON INOCULUM AND FIELD TOLERANCES THRESHOLDS

Unless results of laboratory assays correlate well with field disease development, results of assays are of questionable value. The field tolerance should be known. With a disease requiring a zero tolerance, the assay must be very sensitive. Information about numbers of samples and seeds required per seed lot to obtain statistical results is important (3). Assays must be very specific, regardless of the field tolerance. Information about the anatomy of infected seeds can aid in selecting the best type of assay. For example, with black rot of cabbage, *X. c.* pv. *campestris* is present in the funiculus. This means that there is little need to macerate the seeds. A short washing or soaking (3 min) in a saline, Tween 20 solution to extract the bacteria is very successful (10). However, increasing the time of soaking can increase recovery of *X. c.* pv. *campestris* from some seed lots (C. van Henton, *personal communication*).

One should consider the ecology of the pathogen when using seed assays. Does contamination occur from the vascular system, from external infection, or from external dust? Also, the type of contamination can differ from one lot to another.

Most bacteria are dispensed rapidly in rain or overhead irrigation, but they differ greatly with respect to temperature requirements for disease development. For example, in Florida, two cabbage crops are commonly grown each year, one in the fall and one in the spring. Temperature becomes very important. If temperatures remain cool in late winter and early spring, black rot of crucifers does not become a problem, whereas if the temperature turns warm, serious black rot often occurs. In Idaho, halo blight of beans is most easily detected in young plants in early spring and in mature plants in the fall, when the weather is more conducive to

TABLE 2. Assay methods for seedborne bacteria and indication of whether field tolerances have been tested and established

Method of assay	Pathogen	Tolerance established?
Growing on	*Pseudomonas syringae* pv. *glycinea*	No
	pv. *phaseolicola*	No
	pv. *pisi*	No
	pv. *lachrymans*	No
	Xanthomonas campestris pv. *campestris*	No
	pv. *oryzae*	No
	pv. *phaseoli*	No
Plant injection	*Clavibacter michiganense*	
	subsp. *michiganense*	No
	P. syringae pv. *glycinea*	No
	pv. *phaseolicola*	No
	X. campestris pv. *phaseoli*	No
Agar media	*P. syringae* pv. *phaseolicola*	Yes
	pv. *campestris*	Yes
	pv. *phaseoli*	No
Phage	*C. m.* subsp. *michiganense*	No
	P. syringae pv. *phaseolicola*	No
	X. campestris pv. *oryzae*	No
	pv. *phaseoli*	No
Serology	*P. syringae* pv. *phaseolicola*	No
	C. m. subsp. *michiganense*	No
	X. campestris pv. *campestris*	Yes
	pv. *phaseoli*	No

TABLE 3. Correlation of laboratory assay results to black chaff development in the field[a]

Infected cultivar and seed lot	No. seeds from: Foundation lot	Infected lot	Laboratory assay: Mean cfu *X. translucens*/ml	Field disease: No. plots with black chaff	Disease rating[b]
Bliss	3,000	0	7	0	0.00
1106	2,970	30	79	0	0.00
	2,700	300	266	0	0.00
	0	3,000	1,205	1	0.25
Waid	3,000	0	0	0	0.00
1084	2,970	30	1,170	1	0.25
	2,700	300	42,400	4	2.50
	0	3,000	363,083	4	4.00

[a] Eight replications were made for four mixtures of naturally contaminated wheat seeds of Bliss lot 1106 and Waid lot 1084 with seeds of foundation lot 1090. Four replications of 125 g of seed were assayed by plating washings onto a semiselective agar medium (XTS agar) and four replications were sown in the field 7 days later on 7 May 1983 at Kimberly, ID. Each plot was at least 4.6 m (15 ft) from an adjacent plot. Data taken from Schaad and Forster (11).

[b] Disease rating: 0 = no black chaff; 1, 2, 3, and 4 = one site with 1–2 infected plants, two sites with 1–2 infected plants, three to four sites with 3–4 infected plants, and numerous sites with 3–4 infected plants, respectively. Plants were read on 19 July. Isolations were made from leaves of plants with black chaff symptoms collected at random from each plot. Figures are mean of four plots.

blight development. Symptoms are seldom observed during the warmer, drier summer months. In the Idaho halo blight epiphytotic in 1984, the disease was not discovered until September, just before harvest. No leaf symptoms were observed in most fields. Most infections were found on pods in the wind row.

With halo blight of beans, Walker and Patel found that epiphytotics resulted following primary infection levels of 0.02% (21). Guthrie et al reported that an initial infection of 1 seed per 16,000 could result in complete crop loss (4). One of the most comprehensive studies on using field disease for developing tolerance levels is that of Trigalet and Bidaud (18). They assayed seeds to be planted by growers and established a tolerance level of 1 infected seed per 20,000 for controlling halo blight of bean in France. The tolerance is based on agar isolation and a direct immunofluoresence (IF) assay. Seeds are soaked in tap water at 4 C; after 4 hr, smears are made for IF, and after 10 hr, a sample of liquid is streaked onto King's Medium B agar. Suspected colonies of *P. s.* pv. *phaseolicola* are identified by IF and physiological tests. Seeds from 476 seed lots were assayed over a 6-year period and found to contain a level of infection ranging from 5 infected seeds per 1,000 to 1 infected seed per 20,000. Their results showed that severe epiphytotics resulted from those seed lots containing 5 seeds per 1,000, whereas only a few diseased plants resulted in those seed lots containing 1 infected seed per 20,000. Seed lots containing 5 infected seeds per 10,000 yielded variable results, depending on the season (18).

In England, Van der Plank's (19) infection rate equation ($r = 2.3/t_2-t_1\ [\log_{10} X_2/1-X_2 - \log_{10} X_1/1-X_1]$) has been used to determine tolerance levels for *P. s.* pv. *phaseolicola* (17). Based on 3 years of field plot data, an infection rate of 0.15 and a transmission rate of 10:1 is common in England (17). Therefore, a seed lot with an infection level of 0.025% would not result in a field disease. However, in later studies, a transmission ratio of 2:1 was observed. This illustrates the problems one faces with epidemiological formulas and field data. In England, a green bean crop with a 4% infection at harvest is considered to be a tolerable level. Taylor's results in England (17) agree well with observations by Wharton (23) and Guthrie et al (4) that severe crop losses occur from primary infections of 0.01 and 0.006%, respectively. This would represent seed infection levels of 0.1–0.06% when assuming a seed transmission of 10:1, a level higher than the suggested tolerance level of 0.025%. These results agree favorably with a tolerance of 0.01% for *X. c.* pv. *campestris* (13).

With *X. c.* pv. *campestris*, the causal agent of black rot of crucifer, seedborne inoculum becomes even more important because the crop is often grown from transplants.

The dangers of infected seeds in transplant beds was recognized in the 1960s by the transplant industry in Georgia (J. Ratcliff, *personal communication*). Transplant fields were inspected and certified free of black rot. Because many growers still had trouble with black rot in transplant beds, a project was initiated to work on the development of a laboratory assay. Using seed washings and a selective agar medium, we were successful in detecting 1 infected seed per 10,000 (13). These tests were done by adding infected broccoli seeds to healthy cabbage seeds. The plots were established in Charleston, SC, under optimum conditions for black rot. Black rot was low in plots with an initial infection of 0.01% but high in plots with 0.03% or greater. Therefore, a tolerance of 0.01% was established (13). These tests were based on four replications of 10,000 seeds.

Unlike the work in France (18), our attempts at using IF as a direct seed assay have proven unsatisfactory with *X. c.* pv. *campestris*. Results comparing agar plating and IF assays with field disease showed a positive correlation with the agar plating assay but a negative correlation with the IF assay (9). Of the 24 plots seeded from 12 separate seed lots shown to be IF positive (two replicated plots per seed lot), only one plot resulted in black rot. On the other hand, all plots from seed lots that tested positive for *X. c.* pv. *campestris* by NSCA agar plating assays resulted in black rot in the field. These results clearly support use of agar plating but not IF for issuing a phytosanitary certificate for black rot. The most likely reasons for our poor correlation between IF and field disease are 1) detection of dead cells, 2) nonspecificity of antiserum, 3) inherent specificity problems with IF, or 4) high sensitivity. With a serological assay that does not allow one to prove pathogenicity, there must be a very high correlation between the laboratory assay and field disease. The present method of assaying seed for *X. c.* pv. *campestris* involves plating washings of 10,000 seeds onto NSCA, a general purpose plating medium, and NSCAA and BSCAA, two selective media (7).

Ideally, inoculum thresholds should be established before establishing tolerance levels for planting or quarantative purposes. For example, if seed is to be sown in a climate where the disease is not expressed because of cool temperatures, what is the value of a zero tolerance? The greatest need in the control of seedborne diseases is the development of inoculum threshold data and the correlation of field disease to laboratory assay results.

LITERATURE CITED

1. Clayton, E. E. 1931. Vegetable seed treatment with special reference to the use of hot water and organic mercurials. New York Agric. Exp. Stn. (Geneva) Tech. Bull. No. 183.
2. Dickson, J. G. 1956. Barley diseases. Pages 23-73 in: Diseases of Field Crops. 2nd ed. J. G. Dickson, ed. McGraw Hill, New York.
3. Geng, S., Campbell, R. N., Carter, M., and Hills, F. J. 1983. Quality control for seedborne pathogens. Plant Dis. 67:236-242.
4. Guthrie, J. W., Huber, D. M., and Fenwick, H. S. (1965). Serological detection of halo blight. Plant Dis. Rep. 49:297-299.
5. Jones, L. R., Johnson, A. G., and Reddy, C. S. 1917. Bacterial blight of barley. J. Agric. Res. 11:625-643.
6. Neergaard, P. 1962. Tolerances in seed health testing. A discussion on basic principles. Proceed., Inter. Seed Test. Assoc. 27:386-399.
7. Randhawa, P., and Schaad, N. W. 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. Phytopathology 74:268-272.

8. Schaad, N. W. 1982. Detection of seedborne bacterial plant pathogens. Plant Dis. 66:885-890.
9. Schaad, N. W. 1983. Correlation of laboratory assays for seedborne bacteria with disease development. Seed Sci. Technol. 11:877-883.
10. Schaad, N. W., and Donaldson, R. C. 1980. Comparison of two methods for detection of *Xanthomonas campestris* in infected crucifer seeds. Seed Sci. Technol. 8:383-391.
11. Schaad, N. W., and Forster, R. L. 1985. A semiselective agar medium for isolating *Xanthomonas campestris* pv. *campestris* from wheat seeds. Phytopathology 75:260-263.
12. Schaad, N. W., Gabrielson, R. L., and Malanax, M. W. 1980. Hot acidified cupric acetate soaks for eradiation of *Xanthomonas campestris* from crucifer seeds. Appl. Environ. Microbiol. 39:803-807.
13. Schaad, N. W., Sitterly, W. R., and Humaydan, H. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers in the field. Plant Dis. 64:91-92.
14. Sheppard, J. 1983. Historical perspective of the production of disease-free seed, control and management of bacterial blight of beans in Canada. Seed Sci. Technol. 11:885-891.
15. Smith, E. E. 1917. A new disease of wheat. J. Agric. Res. 10:51-53.
16. Smith, I. M. 1984. Activities of the European and Mediterranean Plant Protection Organization in relation to seedborne pathogens. Seed Sci. Technol. 12:57-58.
17. Taylor. J. D. 1970. The quantitative estimation of the infection of bean seed with *Pseudomonas phaseolicola*. (Burkh.) Dowson. Annals Appl. Biol. 66:29-36.
18. Trigalet, A., and Bidaud, P. 1978. Some aspects of epidemiology of bean halo blight. Pages 895-902 in Station de Pathologic Vegetale et Phytopathologie. Proceed., Fourth Internationa] Conference Plant Pathogenic Bacteria, Anger, France.
19. Van der Plank, J. E. 1963. Plant Disease Epidemics and Control. Academic Press, New York.
20. Walker, J. C. 1952. Diseases of carrot and parsnip. Pages 87-103 in: Vegetable Diseases. J. C. Walker, ed. McGraw-Hill Book Co., New York.
21. Walker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. Phytopathology 54:140-141.
22. Webster, D. M., Atkin, J. D., and Cross, J. E. 1983. Bacterial blights of snap beans and their control. Plant Dis. 67:935-940.
23. Wharton, A. L. 1967. Detection of infection by *Pseudomonas phaseolicola* (Burkh.) Dowson in white seeded dwarf seed stocks. Annals Appl. Biol. 60:305-312.