Sampling for Decision Making in Crop Loss Assessment and Pest Management

Sampling in Seed Health Testing

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ABSTRACT

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Seed health tests are usually performed on a sample of a seed lot; therefore, it is crucial that the test sample be as homogeneous as possible and representative of the lot. Seed sampling procedures appropriate for seed health testing have been developed by seed testing organizations such as the International Seed Testing Association and the Association of Official Seed Analysts. Seed lot size is generally not a constraint when the distribution of contaminated/infected seed in the lot is relatively homogeneous; if the distribution is heterogeneous, increased sampling intensity is required.

Sample size is determined by the damage threshold (intolerable infection level) for the pathogen and the probability of detection desired, commonly 95 or 99%. The probability of detection at a given damage threshold is greater as sample size increases, and this probability and the appropriate sample size can be determined by statistical methods. Most seed health tests utilize qualitative data based on the presence or absence of the pathogen in the test sample, with the lot being rejected if the pathogen is detected in the sample and accepted if the sample is negative.

Additional keywords: seedborne pathogen.

Seed health testing is an essential management tool for the control of seedborne and seed-transmitted pathogens and continues to be an important activity for their regulation and control through phytosanitary certification and quarantine programs in domestic and international seed trade. Seed health testing is also critical for insuring the health of basic seed stocks used for seed production and for plant germ plasm utilized in research and product development. As a consequence of increased product liability (13,19) as well as competitive pressures within the seed industry, seed health has also become an increasingly important quality trait in the marketplace, particularly in vegetable crops. Seed sampling is the first critical activity in a reliable seed health testing program.

Seed health tests are almost always performed on a sample of seed taken from the seed lot. It is, therefore, crucial that the sample used for testing be representative of the seed lot, and this requires standardized sampling procedures. Seed testing organizations such as the International Seed Testing Association (ISTA) (10) and the Association of Official Seed Analysts (AOSA) (6) have developed specific rules and procedures for seed sampling for the evaluation of seed quality traits such as germination and physical purity. Some countries (e.g., Canada [2]) and some states (e.g., California [4]) have included sampling protocols in their seed regulations, and these are generally similar, if not identical, to ISTA or AOSA procedures. Seed health testing programs typically utilize the same standardized seed sampling procedures that are used in tests for germination, physical purity, noxious weeds, and other seed and seed lot characteristics (4,5,10,12).

Mechanics of seed sampling. In commerce, a seed lot is a specified quantity of seed that is physically identifiable by a lot number or some other code and typically meets certain quality standards (3,10). A lot may be composed of seed harvested from a single seed production field or site or from a portion of a field or site. In

some cases, a lot may be composed of a blend of seed from different lots that share acceptable quality traits. Bulk-harvested, un-sized, or ungraded seed is called the mother lot. The process of cleaning seed used for planting, termed processing in the past, is now referred to as seed conditioning by the American seed trade. Depending on the crop type, the conditioned mother lot may be marketed as a single size (e.g., melon, watermelon, pea, and some flowers) or subdivided into smaller lots based on seed size (e.g., cole crops, tomato, field corn, and soybean). Seed lot size varies depending on the crop and, to some extent, its value. Lot sizes of small-seeded crops such as tomato, pepper, cole crops, grasses, and flowers may range from less than a kilogram to several thousand kilograms. Lot sizes of large-seeded vegetable and field crops such as field and sweet corn, bean, pea, corn, and soybean are typically quite large, often in the range of several metric tons.

The objective of seed sampling as stated in the ISTA Rules for Seed Testing (10) is "to obtain a sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot." There are two basic seed sampling mantras. First, it is crucial that the test sample accurately represents the composition of the seed lot as a whole. Second, no matter how accurately an analysis is performed, the results represent only the quality of the sample submitted for analysis. There is also a sampling caveat that is often included with test results in which it is stated that the analysis is based only on the sample tested and does not guarantee that the results are representative of the lot as a whole.

Typical steps used in seed sampling schemes are shown in Figure 1 and are in accordance with AOSA (6) and ISTA (10) procedures. Primary samples of equal size are taken at random from the seed lot and combined and blended for homogeneity to form the composite sample. The submitted sample, which is sent to the testing facility, is drawn from the composite sample. The working sample, on which the actual test is performed, is obtained from the submitted sample after prescribed blending and dividing to ensure sample homogeneity. In some cases, the working sample may be the entire submitted sample or even the composite sample (3,4,6,

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10). These sampling schemes are designed to minimize variability and ensure homogeneity of the working sample.

Although seed health testing programs usually employ the standard seed sampling procedures developed for germination, physical purity, and other tests, there may be exceptions. For example, in Monterey County, CA, sampling of lettuce seed under the county's lettuce mosaic virus indexing regulations (1) differs somewhat from those for drawing official seed samples as prescribed in the California Seed Law (4). The Monterey County regulations require greater sampling intensity (the number and distribution of primary samples) for seed lots in bags, stipulating that a primary sample must be taken from each bag. In contrast, the California Seed Law requires that only up to five bags plus another 10% of the total number of bags in the lot need to be sampled. Specific sampling requirements (e.g., the number of samples to be taken, minimum sample size, prescribed sampling equipment, and sampling intensity) are described in publications by AOSA (6), ISTA (10), and other seed testing groups that have prescribed sampling rules or procedures (2–4). Regardless of details, the key elements in all sampling procedures are random sampling of the seed lot and the blending of primary, composite, and submitted samples to ensure that the working (test) sample is as representative of the lot as possible.

Factors in sampling. Although the goal in seed sampling is to produce a sample of a seed lot for testing that is fully representative of the lot as a whole, there are practical limitations in achieving this. As the seed lot size increases, so does the difficulty in obtaining a truly representative sample. Seed lot heterogeneity (in which the distribution and frequency of pathogen-contaminated/infected seed is not uniform) is one concern. Seed lot heterogeneity may be visible in physical characteristics such as seed discoloration, seed size, or abnormal seed shape. However, for many important seed-borne pathogens, there are no visible differences between diseased and healthy seed. Observations made during seed production and seed conditioning/cleaning can be useful indicators of potential uniformity problems with a seed lot, and these histories should be taken into consideration in decisions on lot uniformity. Practical experience with small-seeded vegetable seed crops indicates that the lack of seed lot uniformity has seldom limited the accuracy of seed health test results as long as the sampling is carefully and consistently done (R. H. Morrison, *unpublished data*). In cases in which the seed lot is known or suspected to be heterogeneous or nonuniform, primary samples should not be pooled until an evaluation of sample uniformity is done (2–4,6,10). For properties such as standard germination, a test of heterogeneity (H value) has been described by ISTA (10). However, the test is apparently not useful for properties that occur at frequencies close to 0%, and some of the most important seedborne pathogens may occur or have the potential to cause epidemics at seed contamination levels far less than 1% (7,8,11,16,17). When heterogeneity is a concern, sampling intensity (the number and distribution of primary samples taken

from individual seed containers or units or the number of units sampled) should be increased over the minimum recommended, but there appear to be no specific guidelines for achieving this. Russell (15) suggested that when pathogen distribution in the seed lot is suspected of not being uniform, the lot should be divided into a series of sublots of a quantity that each could be conveniently handled as a single batch through the seed conditioning process. A sample of the required size to detect the pathogen at the established damage threshold would then be drawn from each sublot and tested independently. Of course, this requires testing a significantly larger amount of seed than normal, but this is likely to be worthwhile, particularly in cases when the risk of seed transmission and the development of an epidemic is substantial.

The maximum size/weight of a seed lot from which a sample can be drawn is typically the same for seed health tests as for other seed quality tests. Several seed testing entities such as AOSA do not specify a maximum lot size/weight from which samples are to be taken (2–4,6). Whereas, under ISTA rules (10), the maximum lot sizes are quite large, up to 10,000 kg for small-seeded crops and up to 40,000 kg for large-seeded crops. There are some exceptions such as the state of Georgia, United States, which has imposed a 500-pound (227 kg) maximum lot size for seed health testing in the certification of crucifer, tomato, and pepper transplants (5). This was presumably done to increase the chance of detecting target pathogens. Although the sampling procedure becomes more critical as lot size increases, a sound and rigorously applied sampling program will optimize the chances of obtaining a representative sample for seed health testing. Experience in the vegetable seed industry, in which standard seed sampling procedures are typically used for drawing both official and quality assurance seed health test samples, suggests that current practices and procedures are satisfactory for seed health testing needs. Some examples of the successful application of seed health testing in the control of major seedborne diseases include lettuce mosaic virus of lettuce (9), black rot (*Xanthomonas campestris* pv. *campestris*) and black leg (*Phoma lingam*) of cole crops (21), and bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) of watermelon (W. Wiebe, *personal communication*).

Sampling errors can occur due to practical limitations in the sampling process (19), resulting in either a false positive or false negative test result. The effect of sampling error is illustrated in an experiment by J. van Bilsen (*personal communication*), in which color-coated *Brassica* seeds were used to simulate contaminated seeds and blended into an uncoated, raw seed lot to give different contamination levels (Table 1). When a series of 50 100-seed subsamples were taken with a standard vacuum seed counter used for black leg (*P. lingam*) testing, the estimated level of contamination was slightly, but consistently, less than the actual contamination level. The coated seed was slightly heavier and smoother than the raw seed and was, therefore, more easily removed from the vacuum head. This illustration has practical implications when pathogen-infected seed may have different physical characteristics from healthy seed. For bacterial blights of bean such as halo blight, caused by

Fig. 1. Typical steps used in sampling seed for the evaluation of seed quality, including seed health testing.

TABLE 1. Comparison of actual seed contamination levels with those obtained when a group of subsamples were taken from four *Brassica* seed lots with different simulated infection levels

Infection level	Simulated infection			
	Lot 1	Lot 2	Lot 3	Lot 4
Actual (%) ^a	0.10	0.50	1.00	5.00
Subsamples (%) ^b	0.06	0.44	0.76	4.54

^a Actual infection levels were established by mixing color-coated seeds to simulate infected seeds into uncoated seed in four 10,000-seed samples. (J. van Bilsen, Bejo Zaden, Holland; *personal communication*).

^b The infection level in the subsamples was the average from 50 100-seed samples taken at random by a standard sampling method used in testing for black leg (*Phoma lingam*). (J. van Bilsen, Bejo Zaden, Holland; *personal communication*).

Pseudomonas syringae pv. *phaseolicola*, infected seed is often shriveled and lighter in weight than healthy seed, and because of this difference, seed health tests for bacterial pathogens of bean are performed on samples of the shriveled, lightweight seed culled out during seed conditioning (20). This does not appear to apply to another important bacterial disease, black rot of crucifers (*X. campestris* pv. *campestris*), probably because infected seed is typically not substantially different from healthy seed in its physical characteristics (R. H. Morrison, *unpublished*). Therefore, each host and pathogen combination must be evaluated case by case to determine if contaminated/infected seed characteristics can impact sampling.

Thresholds, tolerances, and working sample size. Inoculum threshold was defined by Gabrielson (7) as the level of infection on or in seed that will significantly affect disease development and result in economic loss. This definition has been adopted by others (11,12) and appears similar to the concept of damage threshold introduced by Zadoks and Schein (22) and reviewed by Nutter et al. (14). Under these definitions, the threshold would represent an intolerable level of pathogen contamination/infection. Tolerance, in contrast, is a term applied to the permissible, or tolerable, level of seed contamination/infection (11). Thresholds and tolerances have been developed for a number of seedborne pathogens (7,11,16–18, 20,21). Tolerances that have been successfully used to help manage seedborne pathogens include lettuce mosaic virus of lettuce (0/30,000 seeds in California), and the *Brassica* diseases black leg (0/10,000 seeds in the United States and 0/1,000 seeds in Europe) and black rot (0/30,000 seeds worldwide), and anthracnose (*Colletotrichum lindemuthianum*) of snap bean (0/600 in the United Kingdom). As noted earlier, damage thresholds for many of our most important seedborne pathogens are often quite low, in the order of 1% or less, and follow the Poisson distribution (8). However, seed infection/contamination thresholds and tolerances have not been clearly determined for a number of seedborne pathogens (7,10,13, 16,17). For several important seedborne pathogens of vegetable crops, the International Seed Health Initiative (13) and the American Seed Trade Association (R. H. Morrison, *unpublished*) are attempting to compile pragmatic minimum thresholds based on experimental data, as well as empirical evidence and experience, that can be applied to seed in commerce.

It is rarely possible to test every seed in a seed lot for a seedborne pathogen. Only under the special situation of a strict quarantine with an absolute zero tolerance might this be feasible and then only if very small quantities of seed are involved and a non-destructive assay method is available. In practice, for most seedborne pathogens, some tolerance in contamination is allowed or at least statistically implied (8,15), because only a portion of the seed lot is actually tested. Consequently, the following caveat, as mentioned earlier, is applicable for seed health test results: a negative test result does not guarantee that the seed lot is completely free of the specified pathogen(s), only that the sample of seed tested was found to be negative.

The seed contamination/infection threshold is not normally used to determine sampling strategy or method. However, the threshold

may be used to determine the size of the working sample in combination with acceptable statistical probabilities for pathogen detection along with other factors such as direct or indirect testing of seed and test sensitivity (8,19). For direct tests, individual seeds are assayed to determine if they are infected or healthy, and the results can be quantified. In indirect tests, seed sample units (subsamples) of a specific quantity are assayed to determine the presence or absence of the pathogen in the sample unit and, therefore, whether the lot is positive or negative. As an illustration, the number of seeds that needs to be tested using a direct test approach for a few seed contamination thresholds and probabilities of a false negative result are given in Table 2. Table 3 shows the number of seed samples required to achieve different probabilities of a positive test result in the indirect approach, in which subsamples from the lot are tested. In commercial seed health testing programs, indirect testing methods are most often used because of increased time and cost constraints and decreased efficiency associated with the direct testing approach (19).

Decision making and risk management. Information from seed health tests is used in decision making in domestic and international quarantine and phytosanitary seed certification programs as well as for quality assurance and risk management decisions by seed-producing companies and their customers. Essential elements in risk management of seedborne diseases are (i) determination of pragmatic and realistic seed contamination thresholds/tolerances, (ii) assessment of disease potential as a result of seedborne pathogen contamination, (iii) establishment of probabilities for detection of the pathogen on seed, and (iv) determination of the level of risk that is acceptable. If a seed lot does not meet the established tolerance standard for the intended use of the seed, it is rejected. In some instances, a seed treatment may be applied that can eradicate the pathogen or at least provide sufficient control to bring the contamination level of a seed lot to an acceptable level. Further sampling and testing of the treated seed is then required to confirm the efficacy of the treatment.

The seed contamination threshold/tolerance, as well as the probability for detection of a pathogen, may well differ depending on how the seed is to be used (19). Seed health tests should be designed to provide information so that the seed will be sufficiently healthy to meet the requirements for which the seed is intended, i.e., germ plasm, basic seed, or commercial planting stock. For most agricultural applications, a 95% level of confidence for pathogen detection is considered acceptable (15), and this is commonly applied to risk management in seed health testing programs (8,19). However, managing risk for a pathogen with high epidemic potential, particularly if it is likely to be grown in a disease-conducive environment, would probably justify an increase in this confidence level to 99% or possibly higher. Because the level of acceptable risk is not the same for all situations, sound risk management decisions will depend on the purpose for which the seed is to be used as well as the tolerance for risk of those making the decisions. Risk assessment and risk management in seed health is often challenging and difficult, because many of the important factors that influence the outcome, for example, seed inoculum levels, environmental conditions, crop production practices, and the myriad

TABLE 2. The number of seeds to test in seed lots with various threshold levels of infection and the probability of obtaining a false positive result using a direct test approach^a

Threshold infection (%)	Probability (%) of false negative	No. of seeds to test
0.01	5	29,956
0.01	1	46,049
0.05	5	5,990
0.05	1	9,208
0.1	5	2,995
0.1	1	4,603

^a Test in which individual seeds are evaluated and the pathogen determined to be present or absent in each seed (J. van Bilsen, Bejo Zaden, Holland; *personal communication*).

TABLE 3. Relationship between the number of subsamples to test and the probability of pathogen detection for any given seed infection threshold using an indirect testing approach^a

No. of subsamples of <i>n</i> seeds	Probability of a positive test result (<i>P</i> ₊)
1	0.632
2	0.865
3	0.950
5	0.993

^a Tests in which subsamples of a specified seed quantity are evaluated and reported as positive or negative. These probabilities assume a test sensitivity of 100% (8).

of possible biological interactions, are unknown or can only be roughly estimated.

Conclusion. Seed health testing is an important component in disease management for quarantine and phytosanitary certification and is increasing in importance in quality assurance programs for commercial seed for planting, basic seed used for seed production, as well as germ plasm. Seed sampling is key to obtaining accurate seed health test results. Because of the low frequencies with which many important seedborne pathogens occur in seed and the low to very low seed contamination/infection thresholds for economic loss or the initiation of an epidemic, sampling for seed health testing may appear to present special problems. Nonetheless, most seed health testing programs typically employ the same standardized seed sampling procedures that were developed and are used for germination and purity analyses (10,12), and these sampling standards appear to be quite satisfactory in providing accurate and reliable seed health information.

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