

## Some Aspects of Sampling and Statistics in Seed Health Testing and the Establishment of Threshold Levels

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The establishment of acceptable threshold levels of seedborne pathogens requires sampling techniques that are fairly representative of seed lots tested and adherence to acceptable statistical procedures. In this paper, I will discuss some aspects of sampling, the analysis of qualitative data using binomial tables, the use of binomial tables to establish appropriate sample sizes, and the statistical handling of quantitative data in seed health tests.

### SAMPLING

For an appropriate seed health test, the lot from which the sample is taken must be uniform (1). This brings us to the questions of what you can do when you suspect that the pathogen population is not uniformly distributed throughout the seed lot. I do not have a general solution, but I do have a suggestion. Suppose you have bags, bins, or units of seed of such size that you can stir or mix uniformly, a unit of seed no larger than what can be handled in a single batch through a seed conditioning process. For small-seeded vegetable crops this is approximately 454 kg. Then, sample each bag, bin, or unit for a specified sample size. This is equivalent to stratifying or blocking a field where you are determining yield, height, etc. For example, you have a seed lot of 4,540 kg in 10 boxes, each containing 454 kg. Your concern is that the test does not exceed 0 per 10,000 infected seeds. You should take 10 samples of 10,000 seeds, one from each box, and analyze each sample independently. You should not mass the 10 samples and subsample

to one 10,000-seed sample.

The next question that arises is, Is it better to use four samples of size 100 or one sample of size 400? If the criterion is to reject if you find one infected seed, the probabilities are identical. However, I recommend using the four samples of size 100 over one sample of 400, because you can obtain an estimate of the variability (repeatability) in your laboratory. If the critical or threshold value (the number of infected seeds necessary to reject the seed lot) is not zero, the power of the test to reject infected seed lots is greater for multiple samples than for a single sample although the percentage of infected seeds will be the same.

### EVALUATION OF QUANTITATIVE DATA

Where seed health tests are designed to provide data based on health or disease of individual seeds (e.g., percentage of diseased), the data generated are qualitative and are best suited for analysis by binomial distribution (8).

It is the nature of some host-pathogen combinations that a very low level of seedborne pathogens can spread and cause economic loss from the disease. Examples include lettuce mosaic virus (7), black leg of crucifers (*Phoma lingam* [Tode ex. Schw.] Desm. [2]), and black rot of crucifers (*Xanthomonas campestris* pv. *campestris* [Pam.] Dows. [6]). With this situation, it is common to accept the lot if you find no pathogen and to reject it if you do.

You cannot estimate a zero value with a seed health test based on a subsample of a seed lot. In agricultural research, a 95% level of confidence is commonly accepted. At the 95% level of confidence and a zero result for seed sample sizes  $> 100$ , you can estimate that

up to four infected seeds may be found in subsequent tests analyzing the same sample size from the same seed lot. This will be true with minor variations, regardless of seed sample size (e.g., at 100, 1,000, 10,000, or 100,000, you can estimate a 0-4 result at the 95% level). What you gain by increasing sample size is a lowered expected percentage of infection (e.g., 0/100 [4%], 0/1,000 [0.4%], and 0/10,000 [0.04%]). Hopefully, at some point you will arrive at a seed infection level that will not cause economic loss in the field, a threshold level that can be used for disease management.

For some host-pathogen combinations, a higher level (e.g., 20%) of seed infection can be tolerated. Binomial tables (8) can be used to accurately estimate acceptable levels and to determine sample sizes necessary to stay within desired confidence limits. If you observe that pathogen percentages at 15% are safe to plant, you can determine from the binomial tables that a 10% infection level in a 300-seed sample size has a confidence range of 7-14% at the 95% level. Thus, with this sample size, you should require that seed lots be 10% infected to predict < 15% infected seed in the population at the 95% level. In fact, these tables can also be used for exacting 97.5 or 99.5% (one-sided) confidence, because we are only concerned with rejecting the upper limit, not accepting the lower limit. For example, using a binomial table, the 95% confidence for a sample size of 300 when 25 seeds (10%) are observed infected is 7-14% infected. One could say that I am 97.5% confident that the percentage of infected seeds in the population from which the seeds were sampled is 14% or less. The tables may also be used for comparing two different lots by determining whether the confidence intervals overlap. For example, if another lot has 20% infected seeds in a sample size of 300 seeds, the 95% confidence interval is 16-25%; this confidence interval does not overlap with the 7-14% in the previous lot. At the 95% level, you would conclude that a lot with 10% infected seeds and a lot with 20% infected seeds, each from a sample size of 300 seeds, were from different populations. Thus, the binomial tables can be used to estimate the superiority of one procedure (e.g., pathogen assay technique, field practice, seed treatment) over another. This was the method used by Maguire et al (4) to evaluate factors affecting the sensitivity of a laboratory assay for *P. lingam* in crucifer seed. It is likely that computer programs will be available for using desktop computers to figure the exact probabilities or sample sizes for given percentages.

Existing tables can provide practical estimates of sample sizes necessary to stay within desired confidence limits. For example, if one wanted the confidence interval to be less than 5% wide for a population suspected to contain approximately 10% infected seeds, for  $N = 300$  seed, the width is 7% (i.e., 7-14%), but for 1,000 seeds, the width is 4% (i.e., 8-12%). Therefore, a sample size of 300-1,000 seeds would be appropriate.

### EVALUATION OF QUALITATIVE DATA

Where seed health tests are not designed to provide data based

on health or disease of individual seeds, the data are quantitative, not qualitative. Such data come from extraction of pathogenic propagules from massed samples, such as soak extraction procedures to test for *Tilletia caries* (DC.) Tul. in wheat seed (2) and *X. c. pv. campestris* (6) in crucifer seed. However, if a zero result is obtained in 10,000 seeds, for example, this is equivalent to testing 10,000 seeds, each with a zero result, and this figure can be used to estimate the probability that the seed lot contained infected seed (e.g., approximately four infected seeds per 10,000 at 95% confidence). Binomial probabilities do not apply if the observed infection levels in these massed samples are above zero. To estimate the number of pathogenic propagules (e.g., spores and bacterial colony-forming units [cfu]) under these conditions, you must run replicate samples at the level of sensitivity desired (e.g., cfu per 10,000 seeds), define the population as mean and standard deviation, and compare different samples by an appropriate analysis (e.g., analysis of variance).

Regression analysis may be used to establish threshold levels of pathogens in seed lots estimated by the above procedures that will result in economic loss from plant disease in the field (3,5).

### SUMMARY

You cannot predict a zero pathogen count with a test result taken from a sample. You can use binomial tables to predict appropriate sample size when tests yield qualitative data. You should use binomial tables to evaluate qualitative data, but not to analyze quantitative data. However, you can predict possible infection levels with massed data when a zero pathogen count is required.

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