Thresholds, standards, tests, transmission and risks

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Summary

The purpose of seed health testing is to minimise the risk of damaging levels of disease developing in a crop or to minimise the risk of introducing a new disease. The level of the pathogen in seeds which gives rise to an unacceptable risk of disease is often referred to as 'Inoculum Threshold', although the term 'Tolerance Standard' is perhaps less misleading and to be preferred. The risk of a 'significant' epidemic developing is dependant on the rate of transmission from seed to seedling and the rate of disease increase in the crop (both of which are highly dependant on environmental conditions). Transmission of disease from seed to seedling can be described by the 'One-hit' infection model. The relationships between seed health assay results, tolerance standards and disease risks are discussed.

Introduction

There have been a number of reviews covering correlations between seed health test results and field transmission of seedborne pathogens: (Maude, 1996; McGee, 1995; Mink, 1993; Gabrielson, 1988; Schaad, 1988; Stace-Smith and Hamilton, 1988). Therefore this paper is not intended as a definitive review of the literature, but as a personal (and perhaps biased) view of some of the problems of interpreting seed transmission data and the current approaches being taken to solve some of these problems.

Aims of seed testing

Most would agree that there is a continuing need for seed health standards derived from knowledge of pathogen biology and quantitative epidemiology. Over recent decades there has been a great emphasis on the development of 'new' and 'more sensitive' diagnostic methods for particular diseases, but often this has been done without clearly defined targets based on a sound knowledge of the epidemiology of the disease. There is therefore a danger of 'applying technically ingenious methods to the collection of observations that test no hypothesis' (Hewett, 1983)

It is essential to be clear of our aims when carrying out seed testing, these may be either:

(a) To minimise the risk of damaging levels of disease developing in a crop (Certification, Quality control, avoiding litigation)

or

(b) To minimise the risk of introducing a new disease into a region or country (Quarantine)

We should note the use of the term "minimise the risk". It is impossible to completely eliminate risk and so we need to define what are acceptable/unacceptable risks and devise effective methods for determining these risks; also we should not rely solely on seed testing to control the disease, but should aim to back up seed tests with other disease management practices.

Defining the risk

In order to define the risks from seed-borne disease we must first quantify relationship(s) between disease in the crop and amount of pathogen in the seed. This requires quantitative

data on transmission of the pathogen from seed to seedling and the rate of spread or increase of disease in the crop. We also need to define what are unacceptable levels of disease in the crop; these will change with crop use, whether for certification or quarantine purposes. We also need to define what is an acceptable risk of such an unacceptable level of disease.

Determine the risk

We then need to determine the risk: we need to design an appropriate seed health assay and then perform and interpret the seed test.

Thresholds and standards

Many papers on seed health testing use the term 'Thresholds'. The use of this term is misleading for a number of reasons:-

- Disease thresholds determined experimentally are often artefacts of the experiments themselves.
- The word threshold implies some 'magic number' below which there will be no problems, when in fact there is a continuum of risk above and below the 'threshold'.
- Many have been set arbitrarily and are not based on sound epidemiological data.

The use of the term 'standards' is therefore to be preferred as it has no implications other than some arbitrary level agreed by a group of people.

Correlations between seed health assays and disease

There are relatively few examples in the literature where there have been definitive experiments examining the relationship between seed-borne disease levels and disease in the field. Most examples relate to fungal pathogens and a only a few to bacteria and viruses. In most cases the seed test results are reported in terms of the % of seeds infested, and in general the % disease incidence correlates well with seed test results.

However when we critically examine some of these data a number of problems arise:

1. In many cases only limited numbers of seeds were sown (or the numbers were not specified). Thus, there was a minimum transmission rate which could be detected (Table 1). It would therefore be dangerous to base tolerance standards directly on the results of such experiments, and yet, the tolerance standards suggested by the authors are remarkably similar to the minimum detectable transmission rates of the particular experiments. Hence the apparent tolerance standards are an artifact of the experiments themselves.

Table 1. Minimum detectable transmission rates in experiments used to determine 'tolerance standards' for seedborne diseases.

Pathogen/host	Number of seeds sown per plots	Minimum detectable transmission rate
Phoma/brassica	unknown	?
Alternaria/brassica	900	0.3%
Xanthomonas/brassica	40,000	0.008%
Pseudomonas/beans	30,000	0.01%

2. The different infection levels were obtained by diluting a single batch of infected seed with healthy seed. Thus all the infested seeds are from a single population and hence all would have the same mean dose and distribution of inoculum. However, it is clear that transmission is highly related to the inoculum level per seed, as shown by Heald (1921) and Roberts *et al.* (1996), and emphasised by Colhoun (1983)

3. The experiments are often done in only a very limited range of environments. Thus any tolerance standards so-derived are only applicable to the cropping situation or region in which they were obtained.

'One-hit' Infection Model

The relationship between the inoculum dose on individual seeds and the transmission of the pathogen or appearance of primary infections in a crop can be interpreted in terms of the 'One-hit' infection model. This model assumes: that each pathogen cell or propagule acts independently (i.e. each cell is inherently capable of causing infection); that the probability, *w*, of an individual being effective (i.e. giving rise to infection or transmission) is the same for all cells; that the host subjects are homogeneous; and that the potential number of infection sites is large. The probability, *p*, of an infested seed giving rise to an infected plant is:

$$p = 1 - \exp(-w.d)$$

where *w* is the probability of infection for a single cell in the dose *d*. We can rearrange this equation to give:

$$\ln[-\ln(1-p)] = \ln(w) + \ln(d)$$

so that, theoretically, a plot of ln[-ln(1-*p*)] v ln(*d*) should have a slope of one and an intercept of ln(*w*). Unfortunately real life doesn't always seem to fit the theory and we have found it necessary to include an additional parameter, *x*, in the model:

$$p = 1 - \exp(-w.d^{x})$$

Possible explanations of the need for this additional parameter are considered by Roberts *et al.* (1996), but essentially this extra parameter means that as the dose per seed increases the effectiveness of individuals in that dose appears to decrease. Fitting the model to some of the few examples of published data yields the parameters shown in Table 2.

Table 2. 'One hit' model parameters for seed transmission for various host/pathogen combinations.

	w	*x*
Wheat/*Tilletia caries*	0.0006	0.76
Safflower rust	0.010	0.62
Beans/*Pseudomonas savastanoi* pv. *phaseolicola*	0.054	0.18
Brassicas/*Xanthomonas campestris* pv. *campestris*	0.014	0.32
Peas/*Pseudomonas syringae* pv. *pisi* (wet soil)	0.063	0.24
Peas/*Pseudomonas syringae* pv. *pisi* (dry soil)	0.006	0.24

Implications

Clearly the dose of the pathogen on individual seeds is important, but is the distribution? Is there a difference in the risk of transmission between: 1 seed with 100 spores/bacteria or 100 seeds with 1 spore/bacterium?

By way of an example, using model parameters derived for *Xanthomonas* in Brassicas, the probability of at least one infested seed giving rise to transmission was calculated for a constant total inoculum distributed over varying numbers of seeds in a sample of 10,000 seeds (Figure 1). Quite clearly it can be seen that as the inoculum is distributed over increasing numbers of seeds the probability of seed transmission increases significantly. It should be noted that the detection threshold of the current ISTA method (Roberts & Koenraadt, 2005), which now omits a centrifugation step, is 1,500 cfu in a 10,000 seed sample. Clearly with this detection threshold, seedlots carrying inoculum loads well below the threshold would be likely to give transmission if the inoculum was distributed over many seeds.

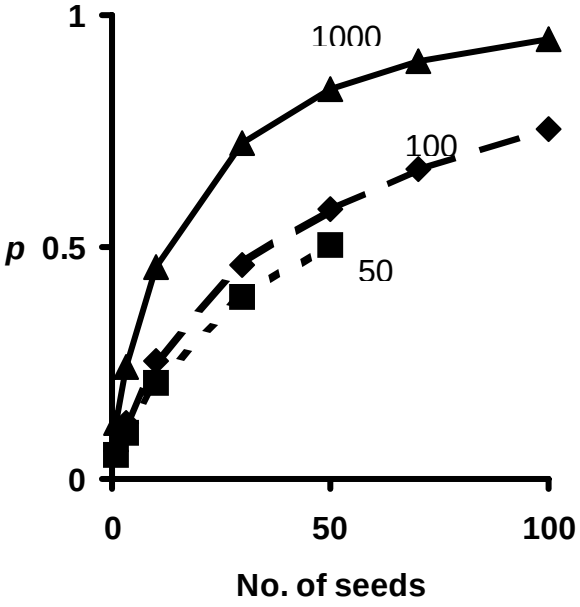

Figure 1. Relationship between probability of transmission, *p*, and the number of seeds over which the inoculum is distributed for *Xanthomonas campestris* pv. *campestris* and brassicas. Each line represents a different total number of bacteria.

Conclusions

There are a number of examples in the literature of good correlations between seed health test results and disease transmission. However, these data have often been obtained in one environment with one seedlot and with limited numbers of seeds sown in each plot. As a result 'tolerance standards' based directly on such experimental results may be artefacts of the experiments themselves.

Most seed tests estimate seed infection in terms of the percentage of seeds infected and the inoculum dose per seed has largely been ignored. However, there is clear data in the literature to demonstrate that inoculum dose has a major influence on disease or pathogen transmission. The environment into which the seed is sown can also have a major influence on transmission.

The parameters of the models fitted to experimentally determined relationships between inoculum dose and transmission imply that the distribution of inoculum may play a critical role in determining the probability of transmission.

Future

It is clear that more information on the distribution of inoculum on individual seeds is needed. There is also a need for models which integrate the apparent % infection and the mean dose per seed, and to examine sensitivity of seed health tests in relation to inoculum dose as well as % infection, if we are to design more effective seed health assays and minimise the risk of disease.

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