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Management of Seedborne Bacterial Diseases

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Introduction

With increasing globalisation of agricultural markets, the removal of trade barriers and the trend for seed companies to amalgamate to form large multinational companies, the movement of commercial quantities of seed both between countries and within countries has greatly increased. Seed is also distributed in smaller quantities for use in trials, for multiplication, for research and for deposition in germplasm collections. As many of the major bacterial plant diseases are primarily seedborne, the opportunities for dissemination of these diseases has never been greater. The control of seed-borne diseases has thus become a major concern for everyone involved in the production, marketing and use of seeds of agricultural and horticultural crops.

Disease management can be considered as the sum of all the actions or control measures taken to limit diseases to below economically damaging levels. We can think of it in terms of reducing the risk of disease. The development of an effective disease management strategy depends on a thorough knowledge of the biology and epidemiology of the pathogen and disease. However, economic considerations will also play a part in the choice of strategy employed.

Management of bacterial diseases presents particular problems compared to fungal and viral diseases. *Bacterial pathogens* are small, effectively invisible and difficult to detect and identify in the absence of disease symptoms. They cannot directly penetrate the plant cuticle and must enter plants via natural openings or wounds. As they require the presence of free water for entry into the plant and are dependant on rain splash for within-crop dispersal, environmental conditions play a major role in the expression of disease and the development of epidemics. Once inside plant tissues they multiply rapidly: a single lesion may contain up 10^9 bacteria. Hence, once disease is established in a crop inoculum is rarely a limiting factor and there is a great potential for explosive increase of disease in conditions suitable for spread and infection.

Control Options

The control measures available for bacterial plant diseases can be divided into four main categories: avoidance, resistance, treatment, cultural practice. These measures can be applied at different levels within the global agricultural ecosystem (international, national, regional and farm) by means of international and national legislation, by market pressures and by farmers and growers themselves. The

particular control options employed and the level at which they are applied is dependent to a large extent on the real or perceived risk to crops and the economic importance of that crop in a particular region or country

Resistance

The successful development and deployment of disease resistance depends on having an understanding of the interactions between host and pathogen. We can think of resistance in terms of race-specific resistance, race non-specific resistance or field tolerance. Race specific resistance is the most well understood and widely used form of resistance.

Whenever sufficiently detailed studies of the host-pathogen interactions in bacterial diseases have been done, pathogen strains can be divided into a number of races on the basis of their reactions with a number of host differentials. This race specific resistance is controlled by the interaction of host resistance genes with pathogen avirulence genes. The presence of matching gene pairs in both the host and pathogen confer resistance, if there is no match susceptibility results. This simple system can lead to complex patterns of interactions as for example in pea bacterial blight caused by *Pseudomonas syringae* pv. *pisi.*

Race specific resistance has been used very effectively to control bacterial diseases in countries or regions where only a limited number of races are present. For example, pea bacterial blight was controlled very effectively in the UK for many years because the main varieties which were grown and multiplied in the UK (Maro and Progreta) were both resistant to the dominant race of the pathogen (Race 2). New 'improved' cultivars were introduced from Europe in 1985 which were not only susceptible to Race 2 but were also infected. The disease is now widespread in the UK.

Although disease resistance would seem to be the most desirable approach to controlling bacterial plant diseases, it is unlikely to be and should not be relied upon as the sole means of control. In commercial breeding programs, other characteristics, such as eating or keeping qualities of produce may be of much greater importance, and in the case of minor crops it may not be economically viable to breed for resistance.

Disease Treatment

Seed

A number of different chemical and physical treatments have been investigated for control of seedborne bacterial diseases. Chemical treatments involving antibiotics or soaking seed in copper-based compound or disinfectants such as hypochlorite have been widely attempted. Physical treatments of seed using dry heat, steam and, most frequently, hot water soaks have also been used.

The effectiveness of both chemical and physical treatments is very dependant on a number of key variables such as the level of inoculum, its location (i.e. whether external contamination or internal infection), seed quality and seed type, crop species and even cultivar. Thus, seed treatments can reduce or minimise infection levels but should never be expected to eradicate the pathogen completely. It is as well to be aware that the effectiveness of a treatment method can only be determined by carrying out seed tests which in themselves have their limitations as we shall see later. Antibiotics are probably the most effective treatment, but their use is not permitted in most countries, due to concerns of possible transfer of resistance to organisms of medical or veterinary importance. In addition if widely used it is likely that resistance could build up rapidly.

A major problem with nearly all seed treatments, is their adverse effects on seed germination and seedling vigour and/or phytotoxicity. With the increasing demands of modern crop production systems for seed of the highest quality and standards of germination, treatments which reduce germination even slightly are no longer acceptable. For example, although hot water treatment of brassica seeds to control *Xanthomonas campestris* pv. *campestris* has been widely used for many years, the associated reduction in germination cannot now be tolerated in expensive seed of F1 cultivars used in module plant-raising systems.

Crop

The options for treatment of bacterial diseases in the growing crop are very limited. There are only a few bactericides available as a result of the much greater emphasis placed on development of fungicides by the major manufacturers.

Although antibiotics are effective and have been formulated for agricultural use in some countries, their use, as stated earlier, is not permitted in most countries. Where they have been widely used their effectiveness has ultimately been limited by the development of resistance.

Copper-based compounds are the most widely used chemicals for control of bacterial diseases in the field. However, as they have a protectant mode of action, frequent applications may be necessary to achieve effective control. Copper is phytotoxic to many plant species and copper resistant bacterial strains are well known. Thus, achieving effective control makes the appearance of toxicity symptoms and the development of resistance more likely.

Cultural practice

Cultural practices can play a vital role in the management of seedborne bacterial diseases. In some cropping systems cultural practices are the only means of managing bacterial diseases. It is essential to consider the whole cropping system: whilst each individual action on its own may appear to have only minor impact on the likelihood of epidemic development, the cumulative effects of a number different

actions can multiplicative. Thus, disease management and crop management strategies need to be developed in tandem. It is often the case that outbreaks of "new" bacterial diseases are the result of some change in cultural practice without regard for the impact on disease.

Crop hygiene

Good general crop hygiene is an essential part of any disease management strategy, and should not be neglected. Bacterial pathogens are often moved from field to field with contaminated farm machinery, it is therefore essential that these are disinfected after use in infected or potentially infected crops. Bacterial pathogens can survive considerable periods of time in dry crop debris, as this is in effect the basis for their success as seedborne pathogens. It is therefore essential that seed trays and plant raising houses are cleaned of plant debris and disinfected and that waste dumps are not allowed to accumulate. Many bacterial plant pathogens have alternate weed hosts which can serve as reservoirs of inoculum, it is therefore essential that these are eliminated from the system.

Rotation is an important practice in the control of most diseases. Most bacterial pathogens survive in crop debris and not free in the soil. The length of rotation is therefore essentially determined by the time taken for crop debris to decay, and any measures which increase the rate of breakdown of debris such as chopping and incorporation are beneficial.

Measures to reduce rate of disease spread

Any measures which result in a reduced rate of disease spread can be of benefit in limiting the impact of bacterial diseases. Farm machinery can play a significant role in spreading bacterial diseases, especially when operations are carried out when crops are wet from dew or recent rainfall, it is therefore important to minimise the number of machinery operations in a crop. Increasing the spacing between individual plants in the field can reduce the rate of plant to plant spread, especially early in crop development. As they are very dependent on water-splash for dispersal, bacterial diseases can be considerably limited in irrigated systems by reducing or eliminating overhead irrigation. In experiments on the transmission and subsequent spread of *Xanthomonas* in brassicas transplants, a capillary watering system reduced disease fourfold compared to overhead watering.

Timing of planting

Transmission of disease or pathogen from seed to seedling during germination and emergence has been directly related to soil moisture levels during this period (Roberts 1992, Roberts *et al*.,1996). Thus, by manipulating sowing time so that seeds are sown into a drier seed bed, transmission can be considerably reduced.

Crop Mixtures

The use of crop mixtures of cultivars or species or both can be an extremely effective means of minimising the impact of bacterial diseases. It is used extensively in subsistence farming systems in less developed regions. For example in central and eastern Africa a number of bacterial and fungal diseases of *Phaseolus* beans are controlled by the use of a complex genetic mixture of landrace varieties carrying different combinations of resistance genes and with different growth habits inter-cropped with Maize.

Disease Avoidance

Disease avoidance through the use of a clean seed policy to exclude inoculum is the most obvious and probably the single most effective means of controlling seedborne bacterial diseases. The are a number of ways of implementing such a strategy: by the imposition of quarantine standards at international or regional borders; by the use of legally required seed certification standards; by means of voluntary seed standards which are effectively driven by market forces and by choice of seed production areas. In all cases the aim is the same: to provide the farmer or grower with seed which is free from the pathogen; and in all cases there is a need for some sort of assay to ensure that seed meets the requirements.

Seed Production Areas

Production of seed crops in areas where climatic conditions are unfavourable for disease development is perhaps an obvious means of avoiding seed infection. Due to the dependence of bacterial pathogens on rain for the development of epidemics, production of seed crops in arid or semi-arid regions can be a very effective means of producing seed with very low levels of infection. This is the basis for production of the majority of bean seed in the US in Idaho. However, as many bacterial pathogens can survive epiphytically with no disease symptoms, the absence of symptoms in seed crops grown under dry conditions may give a false impression of the health of the seed if based solely on visual field inspection. It is therefore vital that the health of seed produced in dry climates is assured by seed health assays designed to detect appropriate tolerance standards.

Quarantine

Most countries have some form of quarantine regulations aimed at preventing the introduction of non-indigenous seedborne diseases. The simplest and probably most effective implementation is to totally prohibit importation of seed and other plant material of the susceptible plant species. It is more usual however that regulations require that seed is only imported from countries or regions which are known to be free of the disease or to only import seedlots which are known to be free from disease.

Quarantine regulations can be very effective and have been used successfully in many instances. However, to be successful the regulations need to be effectively enforced and have a sound epidemiological basis. There is therefore a need for sensitive detection methods and as no assay can ever be 100% reliable there is a need for backup procedures to detect and eradicate escapes.

Quarantine procedures may fail for a number of reasons: problems of enforcement; lack of understanding of the pathosystem or statistics by regulators; inadequacy of field inspection compared to seed testing; a lack of suitable and sensitive detection methods; the misconception that a negative test result means that the seed is healthy; inadequate tolerance standards; lack of knowledge of biology/epidemiology. Pea bacterial blight in the UK provides an example of a failure to control a disease using quarantine regulations.

Pea bacterial blight was classified as a non-indigenous disease in the UK in 1953. The quarantine regulations for import of pea seed into the UK required that the seed was free from the pathogen and was accompanied by a phytosanitary certificate stating that either the disease had not been present in the source region for at least 10 years or that no blight had been found during at least one official inspection since the last complete cycle of vegetation. Although there was no obligatory requirement for a seed test, these regulations remained effective when the amount of seed imported was relatively small, was generally produced in drier climates and the main cultivars grown were in any case resistant to the dominant race of the pathogen. However, following a dramatic expansion in the crop and subsequent commercial pressure for new cultivars and demand for seed, the disease was introduced in 1983-84 with imported seed and seen in crops in 1985. Obligatory testing was started in 1987, but it was by then too late. The disease is now widespread and statutory controls were removed in 1993.

Certification and Voluntary Standards

The aim of seed certification and voluntary standards is to ensure that seeds reach set standards of varietal and physical purity and freedom from disease. Unlike quarantine where the aim is to ensure that seeds are completely healthy, some level of disease may be acceptable. To achieve this appropriate tolerance standards for particular disease need to be set and then seed tests designed to achieve those tolerance standards.

Tolerance Standards

The setting of appropriate tolerance standards for seed health is one of the major problems for bacterial seed pathologists. However, there has been relatively little effort in this area of plant bacteriology compared to the design and development of detection methods. As a result, tolerance standards have in many cases arisen rather arbitrarily or incidentally out of the design of particular assays rather than

being based on the epidemiology of the disease in question. This is entirely inappropriate and it is essential that this bias is redressed: tolerance standards should come first, test systems should then be designed to detect those levels.

In setting tolerance standards it is essential to take a pragmatic view of the commercial reality: there is little point in setting a tolerance standard which is impossible to achieve and would leave farmers without any seed supplies or would lead to inflated seed prices. Athough they should ideally be based on the epidemiology of the disease there must be a balance with the need to maintain seed supplies.

The development of tolerance standards requires epidemiological models driven by: transmission from seed to seedling; rate of spread during plant raising; rate of spread in the field; tolerable level of disease/crop loss at harvest. The data required is perhaps best obtained by means of empirical experiments.

In principle there is no need to set a single tolerance standard: they could vary according to the aims of the standard (quarantine, certification, voluntary), the purpose of the crop (seed crop, ware crop), the cropping system (transplanted, direct drilled), and the environment. There are however only a few examples where tolerance standards have been determined for bacterial diseases based on epidemiological data: beans (UK), 0.025%; spring peas (UK), 0.1%; direct-drilled brassicas (US), 0.01%.

Design of Seed Health Assays

Methods for detecting bacterial pathogens in seeds generally have a number of common features. Following sampling and, sometimes, division into sub-samples, there is usually an extraction procedure during which the pathogen is released into liquid medium. The presence of the pathogen in the extract is then determined by a number of different methods, for example direct plating on selective agar media, immunofluorescence or molecular methods. With the traditional plating methods there is usually a further confirmatory identification step. Precise methods vary between laboratories, for different pathogens and seed types and will be subject to different levels of sensitivity and detection thresholds.

Assays for bacterial diseases can be divided into two types: qualitative and quantitative. Qualitative assays are the most common where the aim of the test is to determine whether a particular seed lot is acceptable or not. In quantitative assays, the aim is to obtain an estimate of the infection level in order to make management decisions or for research purposes. I shall only deal here with qualitative assays, further discussion of the design of quantitative assays can be found in Roberts *et al.* (1993).

No seed health assay can ever guarantee that a seed lot is completely healthy, thus there is always a need for tolerance standards, even in the quarantine situation where the total exclusion is the

aim. As tolerance standards for bacterial diseases are relatively low and tests are usually expensive and time consuming it is not feasible to test seeds individually. Seeds are therefore tested as bulk samples. The main design problems are to determine how many seeds should be tested and to minimise the number of samples. The theoretical basis for the design of seed assays for bacterial diseases is the binomial probability model:

$$p_c = 1 - (1 - \theta)^n$$

where p_c is the probability that an infected seed is present in the sample, θ is the proportion of seed infected and n is the sample size.

There are a number of assumptions implicit in this model:

1. The seeds which are tested are a random sample from the seedlot
2. Each seed can be classified as either healthy or infected
3. Each seed, regardless of health status, has an equal chance of being in the sample
4. The sample size is small relative to the lot size

In practice satisfying these assumptions can be problematical, but they represent a practical starting point. All assays are subject to two type of errors: false positives, when an acceptable seedlot with an infection level below the tolerance level is rejected (probability α) and false negatives, when a seedlot with an infection level above the tolerance level is accepted (probability β). Most seed health assays aim to minimise β, the probability of a false negative, but this must be balanced with the need to maintain seed supplies.

Simplest case

In the simplest case where detection of infected seeds in a sample is always possible, i.e. the test sensitivity is 1, and the probability of a positive test result, p_+, is the same as the probability of an infected seed being present in the sample, i.e. $p_+ = p_c$, it is a simple matter to rearrange the binomial equation to obtain the sample size n in terms of the specified tolerance level θ_{nt} and the required probability of a false negative β:

$$n = \frac{\ln(1 - p_c)}{\ln(1 - \theta_{nt})} = \frac{\ln(\beta)}{\ln(1 - \theta_{nt})}$$

Examples of the samples sizes required are shown in the table for a 95% probability of detection (β = 5%). It should be noted that if detection is always possible there is no need to test more than one sample to achieve the tolerance standard. However, as can be seen in the figure, there is also a

significant probability of rejecting seedlots with infection levels well below the tolerance level, i.e. false positives. In the quarantine situation this may not be a problem as it effectively means that there is a reasonable margin of safety in the testing program. In routine quality control, however, it becomes more important not to reject acceptable seedlots. The only means of improving the discriminatory power of the assay is to test more than one sample of seed (see Ridout and Roberts 1997). Therefore the benefits in terms of costs and simplicity of only testing a single sample must be balanced against the probability, α, of rejecting too many acceptable seed lots. Alternatively a lower probability, 1-β, for detecting the tolerance level could be accepted, reducing the number of seeds which need to be tested.

More difficult case

In the more difficult case where detection is not always possible, i.e. $p_+ \neq p_c$, then probability of obtaining a positive result (p_+) is reduced by a factor p_s, the probability of detecting an infected seed in the sample, i.e. $p_+ = p_s.p_c$ (Geng et al. 1983).

This problem can be dealt with simply by determining the maximum sample size in which an infected seed is certain to be detected and dividing the same number of seeds as determined previously into sub-samples of this size or smaller. A negative result from all such sub-samples is equivalent to obtaining a negative result for the original sample size and the criterion for rejecting a seedlot is: to reject it if one or more sub-samples gives a positive result. The probabilities of false positives, α, and false negatives, β, are then precisely the same as if the sub-samples had been tested as one combined sample.

Alternatively, if the probability of detecting an infected seed, p_s, is known, it is possible to calculate the number of samples, k, of size n, which need to be tested to meet pre-defined tolerance levels using the following formula:

$$k = \frac{\ln(\beta)}{\ln[1 - p_s.p_c]} = \frac{\ln(\beta)}{\ln[1 - p_s(1-(1-\theta_{nt})^n)]}$$

However, it must be borne in mind (when the criterion for rejecting a seedlot is set as at least one sub-sample giving a positive result) that as the number of samples increases, the probability, α, of rejecting a seedlot with an infection level below the tolerance level increases. When multiple samples are tested, it may be more appropriate to set the rejection/acceptance criterion to be something other than the rule of at least one positive sub-sample to reject a seedlot e.g. at least one negative sub-sample.

Another solution, especially if the probability of detecting an infected seed, p_s, is relatively close to one, would be to accept a lower probability for 1-β, the probability of rejecting a seed lot with a non-tolerable level of infection.

Conclusions

Due to their sporadic nature, resulting from a dependence on particular weather patterns for the development of severe epidemics, it is often easy to become complacent about the threat of bacterial diseases in particular regions. Nevertheless a pathogen may still be present in the absence of disease symptoms, insidiously spreading both within and between crops, awaiting a particular set of favourable weather conditions, when explosive disease development may occur from relatively low inoculum levels. As there are limited options for control of bacterial diseases once present in field crops, it is vital to continue to have coherent disease management strategies in place even when the threat of a particular disease has apparently declined. Such strategies should be based on prevention rather than cure.

In the case of seedborne diseases it is obvious to target seed with a "clean seed" policy. Field inspections of growing seed crops are almost always inadequate as a means of ensuring seed health status and therefore it is essential to have a programme of seed health testing. This is most effective when implemented at national and regional levels to ensure that the efforts of growers, plant raisers or seedsmen who play strict attention to disease management are not undone by a few less scrupulous operators.

To be effective it is essential that tests are designed to achieve epidemiologically defined tolerance standards. Setting of tolerance standards requires considerable research effort, but is an essential part of developing a management strategy for any seedborne disease.

Resistance should ideally be the long term goal, but requires a detailed understanding of host-pathogen interactions, and may not be an economically viable option for minor crops. Finally, the role of good crop husbandry and appropriate cultural practices should not be neglected or underestimated in the continuing battle against seedborne bacterial diseases.

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