Inoculum Thresholds of Seedborne Pathogens

Fungi

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One pollen grain can fertilize one ovule. One fungal spore can cause a plant disease, but it often takes thousands of fungal spores to establish a parasitic relationship resulting in disease. The relationship is similar in threshold levels of seedborne disease. For example, Gabrielson et al (15) observed that no disease occurred in a field trial when crucifer seed had 0.6% or less infection with *Phoma lingam* (Tode ex Fr.) Ces. & de Not.

Inoculum thresholds of seedborne pathogens are the levels of infection on or in seed that will significantly affect disease development and result in economic loss. The threshold level must be zero for a disease that is not in an area protected by an established quarantine. The sampling methods routinely used for seed health testing cannot predict a zero level and are not suitable for quarantine purposes. However, for disease management purposes, some infection percentage above zero, a threshold level predicting no effect, needs to be established based on field experience with seed known to contain different infection levels.

To establish a threshold level, the amount of seed infected must be determined. Ideally, the seed testing method should be sensitive, quick, inexpensive, accurate, and reproducible. A number of methods are used in seed health testing for seedborne pathogenic fungi, including visual field inspection, direct examination of seed, examination of seed washings, direct grow out of seedlings, blotter tests, culture techniques with selective media, and biochemical tests.

Seed health tests for specific host-pathogens have been developed and evaluated by the Plant Disease Committee (PDC) of the International Seed Testing Association (ISTA). These methods have been published as the Handbook on Seed Health Testing published in the Proceedings of ISTA in 1965 (22). This contains a number of working sheets, each dealing with one pathogen on one host. A revised handbook is currently being published by ISTA (28). Neergaard (21) summarizes and comments on many methods. In addition, seed testing methods are

widely scattered in the literature published by plant pathologists concerned with specific host-pathogen combinations.

In any test, it is important to guard against both false positives and false negatives. An example of a false positive is the identification of a dead fungal structure (e.g., a pycnidium of *Septoria apiicola* Speg.), or isolation of a strain (e.g., *P. lingam*) with low virulence, or a morphologically similar nonpathogenic fungus (e.g., *P. herbarum* Westend. instead of *P. lingam*). A false positive can be avoided by including a pathogenicity test. A false negative occurs when the conditions of a test fail to reveal a virulent pathogen in the seed. This can be guarded against by including a known infected control in each test. Also, as discussed by Russell, it is also important that sample size and replication be adequate, to measure significant amounts of infection or infestation.

Threshold levels must be developed for the average environmental conditions in which seed is sown. Threshold levels will be influenced by all factors affecting the epidemiology of each host-pathogen combination, such as inoculum level, host susceptibility, pathogen virulence, environment, biology of the pathogen, potential for secondary spread, cropping practices, other sources of inoculum, and relationship of inoculum to seed, each of which will be discussed in this paper.

The relationship of the pathogen to seed. Baker and Smith (9) categorized the various relationships between seed and pathogens. Pathogens such as *Ustilago nuda* (Jens.) Rostr., the cause of loose smut, are carried within the embryo, and a high proportion of infected seed will develop into infected plants. Other pathogens are carried with seed lots as dormant structures, with trash, or on or in seed coats. This inoculum must hydrate, activate enzymes, grow, reproduce, attack the seedling, and fight its way to the soil surface before it can become effective primary inoculum contributing to secondary spread. The soil may be an inhospitable place for some of these pathogens, such as the leaf blighters (e.g., *S. apiicola*), which can survive only a short time in soil in the absence of host tissue. Some seedborne pathogens may rot infected seed before the seedling reaches the soil surface, and primary inoculum is effectively buried. For most pathogens in this category, much less inoculum reaches the soil surface to become infection foci than would be indicated by seed health tests.

For example, wire stem disease of cole crops caused by *Alternaria brassicicola* (Schw.) Wiltshire may be on the surface or below the seed coat. In glasshouse studies, Maude and Humpherson-Jones (19) reported that seedling infection was closely correlated with internal seedborne inoculum. Bassey and Gabrielson (12) also found that at 20 C, wire stem disease was most closely associated with deep-seated inoculum. However, at 30 C, wire stem disease was better correlated with total inoculum.

Inoculum level. Theoretically, one infected seed can give rise to one infected plant, but this is generally not the case under field conditions. A Brussels sprout seed lot naturally infected with *P. lingam* was diluted with clean cabbage seed to obtain the different percentages of infection. This seed was planted in the field in Wisconsin. Plots received at least 2.5 cm of rainfall or irrigation every 5 days. Percentage infected plants was recorded after 6 wk. No disease occurred unless seed infection was $> 0.6\%$ (Table 1). The inoculum threshold in this experiment was between 0.6 and 1.5%.

TABLE 1. Blackleg disease in cole crops grown from seed with different inoculum concentrations

Seed infection (%)	Infected plants[a] (%)
6.0	2.6
3.0	4.6
1.5	1.8
0.6	0
0.3	0
0.15	0
0	0

[a] After Gabrielson et al (15).

Host susceptibility. Heald (16) conducted a classic experiment in 1921 that illustrates the effect of host susceptibility on inoculum threshold level in bunt of wheat caused by *Tilletia caries* (DC.) Tul. Equal weights of spores were added to seed lots of resistant 'Marquis' and susceptible 'Jenkins Club' wheat cultivars and planted in the field. The inoculum threshold level for the resistant 'Marquis' was between 542 and 5,043 spores per grain, whereas for the susceptible 'Jenkins Club', it was 104 spores per grain (Table 2). Thus, threshold levels will be higher for highly susceptible cultivars than for relatively resistant ones.

Pathogen virulence. Within the definition of virulence as the relative capacity to cause disease, isolates of a pathogen can range from avirulent to virulent. Often, seed health tests do not determine virulence. Virulent strains are of primary concern to pathologists. For example, several investigators have reported strains of varying virulence in *P. lingam* (14). The relationship between these strains is unknown and virulence can be determined by seedling inoculation only. Bonman et al (13) demonstrated that only the most virulent strain was able to spread and damage cabbage plants in the field in Wisconsin.

Environmental factors. Two environmental factors, temperature and moisture, profoundly affect inoculum threshold levels. Although pathogenic fungi can be demonstrated by various tests to be on or in seed, environmental factors will be critical in determining whether infected seed become infected seedlings and a primary source of inoculum. What may become a disastrous epidemic in one area may be insignificant in another under other conditions. For example, in the 1973 blackleg epidemic, the same infected cabbage seed planted in plant beds that was associated with disaster in midwestern and eastern states caused no problems in the cool Pacific Northwest.

In another case, Bassey and Gabrielson (11) observed that temperature affected the percentage of wire stem disease of cabbage seedlings grown from seed naturally infected with *A. brassicicola*. As temperatures rose and became more favorable for the pathogen, disease appeared and then became more severe (Table 3). The lower limiting temperature for seed transmission in the seed lot with 98% infection was 10–15 C and for two lots with 77 and 33% infection was 15–20 C. The lower temperature limit for the seed lot with 98% infection reflected a deeper-seated seed infection and less sensitivity to a less favorable temperature for infection.

In a classic experiment that profoundly affected the location of the vegetable seed industry, Walker (29) demonstrated that

TABLE 2. Bunt of wheat on resistant 'Marquis' and susceptible 'Jenkins Club' wheat varieties following artificial infestation with different concentrations of smut spores[a]

Spores (g) added per 100 g seed	Spores per grain		Smutted heads (%)	
	'Marquis'	'Jenkins Club'	'Marquis'	'Jenkins Club'
0.00	104	104	0	2
0.01	542	533	0	6
0.1	5,043	5,333	2	33
1.0	59,229	65,229	8	93

[a] After Heald (16).

TABLE 3. Wire stem of cabbage seedlings in growth chambers at different temperatures grown from seed naturally infected with *Alternaria brassiciola*[a]

Seed lot	Seed infection (%)	Wire stem disease (%) Temperatures (C)				
		10	15	20	25	30
X	98	0	12	22	44	85
Y	77	0	0	9	20	33
Z	33	0	0	6	3	8
Radial growth (mm)		29	53	67	71	44

[a] After Bassey and Gabrielson (11).

epidemic development of blackleg in cabbage seedbeds depended on rainfall. When he covered cabbage seedbeds during rains in Wisconsin, plant infection was reduced (Table 4). Although disease appeared in covered plots, it did not spread or cause serious loss.

TABLE 4. Percentage cabbage blackleg in seedbeds in Wisconsin protected from and exposed to natural rainfall[a]

Lot no	Infection (%)	
	Protected	Exposed
1	0.5	28.7
2	2.1	81.1
3	4.4	49.8

[a] After Walker (29).

Biology of the pathogen. Biology of the pathogen can affect the importance of seedborne inoculum and its threshold level. The pathogen may have more than one means of survival and spread.

TABLE 5. Blackleg disease on oilseed rape from healthy and infected seed treated with an effective eradicant[a]

Seed	Seed treatment	Seedling infection (%)	Disease severity rating at harvest
Infected	−	4.6	16
Healthy	−	4.6	22
Infected	+	3.6	16
Healthy	+	4.4	19

[a] After McGee (20).

TABLE 6. Tolerances for seedborne fungal pathogens

Host	Pathogen	Tolerance in seed (%)	Country	Reference
Tall fescue	*Acremonium typhinum* Morgan Jones & W. Gamms	0–50 seed	Alabama, USA	(10)
Flax and linseed	*Alternaria* spp., *Colletotrichum lini* (Westerdijk) Tochinai, and *Fusarium* spp.	5% basic and certified	UK	(5)
Broad beans	*Ascochyta fabae*	0/600 basic	UK	(6)
Field beans	*A. fabae*	0.1% basic, 0.2% certified basic and certified	UK	(3)
Pea	*Ascochyta pisi* Lib.	6%	Canada	(21)
Green peas	*Ascochyta* spp.	0/200 basic and certified if <20/200 treat seed	UK	(6)
Dry peas	*Ascochyta* spp.	<7% accept, 8–15% accept after treatment, 16% and higher reject	UK	(7)
Sunflower flax and linseed	*Botrytis* spp.	5% basic and certified	UK	(4)
Soybean	*Cercospora sojina* Hara, *C. kikuchii* T. Matsu & Tomoyasu, *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *sojae* (Lehman) Wehmeyer *Colletotrichum dematium* (Pers. ex Fr.) Grove, *F. truncatum* (Schw.) Arx.	4%	Mexico	(26)
All seed	*Claviceps purpurea* (Fr.)Tul.	0.05% weight ratio sclerotia to seed	Japan	(25)
Cereal seeds	*C. purpurea*	1 piece/500 g basic, 3 piece/500 g certified	UK	(3)
Snap beans	*Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav.	0/600 basic	UK	(6)
Cereal seed	*Fusarium* spp. *Helminthosporium sativum* Pam., King & Bakke	>15% treat seed	Denmark	(24)
Pea	*Mycosphaerella pinodes* (Berk. & Blox.)Vestergr.	2%	Canada	(21)
Beet	*Phoma betae* (Oudem.) Frank	0/200 basic treat infected lots	UK	(6)
Flax	*Phoma exigua* Desm. var. *linicola* (Naum. & Vass.)Mass.	1% basic and certified	UK	(5)
Brassicas	*Phoma lingam*	0/1,000 basic treat infected lots	UK	(6)
Crucifers	*P. lingam*	1/10,000	USA	(14)
Pea	*Phoma medicaginis* Malbr. & Roum. var. *pinodella* (L.K. Jones)Boerema	2%	Canada	(21)
Barley	*Pyrenophora* graminea and *P. teres*	>5% treat seed	Denmark	(18)
All seed	*Sclerotinia sclerotiorum* (Lib.) de Bary	0.01% weight ratio	Japan	(25)
Sunflower	*S. sclerotiorum*	10 pieces/1,000 g basic and certified	UK	(5)
Swede rape	*S. sclerotiorum*	10 pieces/100 g basic and certified	UK	(5)
White mustard	*S. sclerotiorum*	5 pieces/200 g basic and certified	UK	(5)
Turnip rape	*S. sclerotiorum*	5 pieces/70 g basic and certified	UK	(5)
Celery	*Septoria apiicola* and *Phoma appiicola* Kleb.	basic and certified treat infected lots	UK	(6)
Cereal seed	*Septoria nodorum*	>5% treat seed	Denmark	(24)
Wheat	*Tilletia caries* and *T. foetida* (Wallr.) Liro	50 spores/grain	Austria	(21)
Wheat	*T. controversa* Kühn	30 spores/grain, 30–100 spores/grain + treatment	Austria	(21)
Barley	*Ustilago nuda*	0.2%	Denmark	(21)
Barley	*U. nuda*	0.1% basic, 0.2% certified	UK	(3)
Wheat	*U. tritici* (Pers.) Rostr.	<0.5% healthy, >2.0% reject, <2.0% >0.5% treat with carboxin	India	(1)
Wheat	*U. tritici*	<1 plant/5 sq. rods, reject below this, treat with carboxin	California USA	(2)
Wheat	*U. tritici*	>0.2% (embryo test), reject <2.0%, treat with Vitavax	Utah, USA	(27)

For example, blackleg of crucifers has both a pycnidial stage, *P. lingam*, and a pseudothecial stage, *Leptosphaeria maculans* (Desm.) Ces. & de Not. *P. lingam* sporulates on living tissue and spreads a limited distance by splashing water. Walker (29) commented that the only significant secondary spread was in seedbeds in which plants are closely spaced. On the other hand, airborne ascospores of *L. maculans* are found on residues of the previous crop. These ascospores are thought to be the important source of inoculum for epidemic disease development of blackleg in oilseed rape (20). McGee (20) (Table 5) demonstrated that seedborne inoculum, which is important in transplanted cole crops (Tables 1 and 4), had no effect on the severity of blackleg in oilseed rape when airborne ascospores from residues of a previous crop was present. Thus, the inoculum threshold for blackleg disease in transplanted cole crops cannot be used for blackleg disease of direct seeded oilseed rape.

Potential for secondary spread. The potential for secondary spread in the field affects inoculum threshold levels and the ultimate severity of disease. For simple interest diseases with no repeating cycle of infection, such as the seedling infecting smuts, field disease will be closely related to initial seedborne inoculum level (23). For compound interest diseases with repeating cycles of inoculum production and reinfection, such as blackleg of crucifers and late blight of celery, a relatively low level of seedborne inoculum can spread in seedbeds and result in severe disease losses.

For simple interest diseases, a finite level of seed infection can be tolerated. Where secondary inoculum can spread rapidly, the threshold level of seed infection must be very low.

Cropping practices. Cropping practices affect inoculum threshold levels. When the same cabbage seed lots that were associated with severe losses in transplant beds in 1973 were direct seeded, there were no reported losses (14). This is consistent with McGee's (20) observation that seedborne inoculum is not important in oilseed rape, which is also direct seeded.

Others sources of inoculum. Inoculum may come from other sources, such as weed hosts or infested residues, as cited above. It is difficult to evaluate the relative contribution of each source of inoculum on yield reduction when both seed- and fieldborne sources of inoculum are present. However, Babadoost and Hebert (8) measured a yield reduction for wheat glume blotch caused by seedborne *S. nodorum* (Berk.) Berk. in the presence of a field source of inoculum. In such cases, it is still necessary to establish threshold levels of seedborne inoculum.

Established inoculum threshold levels. Threshold levels should be established by correlation between infection levels based on seed health tests and disease ratings in field plantings of this seed. Such correlations have been made for some of those reported in Table 6. Jørgensen (18) compared the percentage of diseased barley plants in the field with percentage seed infection by *Pyrenophora graminea* Ito & Kuribay and *P. teres* Drechsl. After testing 138 seed lots over a 2-yr period, he recommended that seed lots with less than 5% infection not be treated. Hewett (17) evaluated the effects of different percentages of *Ascochyta fabae* Speg. on seeds of *Vicia faba*. Results from field studies of 90 seed samples over a 6-yr period established infection standards of 0 per 1,000 infected seed for basic seed and 2 per 1,000 for certified seed. Rennie and Seaton (23) reported a good correlation between the proportion of embryos infected with *U. nuda* and field occurrence of loose smut of barley. It is doubtful that all tolerances were established by field correlations (e.g., *Sclerotinia sclerotiorum* [Lib.] de Bary [Table 6]).

When a zero tolerance for a pathogen is established, it is hoped that the seed will be pathogen-free. However, any seed health test evaluating individual seed based on sampling a portion of a seed lot can never predict that the seed lot will be pathogen-free, as discussed by Russell, because of errors inherent in the sampling process. As the sample tested grows larger, the predicted infection percentage becomes lower, but never zero. For this reason, a seed health test is not a suitable method of enforcing a quarantine. It can, however, define an inoculum tolerance level for disease management.

Little work has been done to establish tolerance levels where effective chemical eradicant treatments are used. In Scotland, no attention has been paid to inoculum thresholds of seedborne pathogens on cereals apart from *U. nuda* because they are controlled by routine seed treatment with mercury fungicides (W. J. Rennie, *personal communication*).

In some cases, the severity of damage has dictated caution in the face of disastrous losses and threatened lawsuits. In the 1973 blackleg epidemic, pathologists from affected areas requested a tolerance of 0 in 100,000 seed. Ultimately, a tolerance of 0 in 10,000 seed was negotiated as a reasonable compromise. Data developed later (15) determined that 6 infected seed per 1,000 could be tolerated. At 0 per 10,000 (predicting 4 per 10,000) at the 95% confidence level, we had established a 10+-fold safety factor.

Seed treatments with some fungicides are becoming part of the certification process (e.g., carboxin for loose smut [2,27]). It is less likely that eradicant treatments will be effective on seed lots with high infection levels. This is recognized in the standards for *Ascochyta* spp. in dry peas and *U. nuda* in wheat shown in Table 6 (1,7).

Table 6 provides a partial list of tolerances established for seedborne pathogenic fungi. Establishment of valid tolerances for local areas coupled with accurate seed health tests provides a powerful disease management tool that can minimize the use of fungicides in crop production.

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