Fungicide Distribution and Disinfection Efficiency in Seed Treatment

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Relations between dose of fungicide and disinfection efficiency in seed treatment are formulated by chemical kinetic procedures. The theory is developed on the assumption that diseased seedlings are caused by "disease centers" Poisson-distributed between the kernels of seed. The rate of disinfection of these disease centers is governed by a rate-determining step in one of the physical and chemical processes involved. The reaction order is thus obtained by analysis of biological test results. These relations are used to estimate the drop in disinfection efficiency to be expected for unevenly treated seeds as compared to uniformly treated seeds. Calculations show that distribution parameters reported for liquid seed treatment (Panogen process) are satisfactory and produce a negligible drop in disinfection efficiency.

IN MODERN SEED TREATMENT the volume of the seed dressing is extremely small compared to the quantity of treated seed. Accordingly the distribution of the active principle on the treated seed has become a matter of major interest. The distribution of the fungicide was determined by a radioactive tracer method in a recent study (*9*) of the mechanism of liquid seed treatment (Panogen process). Since then a new chemical method has been devised for accurate determination of the mercury content of single kernels of treated seed (*8*). Neutron activation analysis can also be used (*10*). Statistical treatment of data obtained by analytical methods gives a precise description of the fungicide distribution (*9*). The present paper relates the disinfection efficiency as observed in the biological tests with the distribution pattern of the fungicide as evaluated physically and chemically.

Theory

Distribution Parameters. When fungicide contents of single kernels of treated seed are plotted to give a frequency curve, normal distribution is indicated (*9*). (Actually inspection of very large samples shows that the distribution is slightly skewed.) The population of analytical results—e.g., expressed as micrograms of mercury per kernel, or micrograms of mercury per unit kernel area—is characterized by its average, Q, and its standard deviation, σ_Q. This distribution is conveniently described by means of its coefficient of variation, σ_Q/Q, called the spreading error.

A few per cent of the treated kernels may carry larger amounts of fungicide because of damaged seed coat and/or because of unfavorable mixing conditions. These kernels, called R-kernels, are separated from the group of normal kernels, N-kernels, with the borderline at twice the average fungicide content. The R-kernel population is characterized by three parameters: (1) R = number of R-kernels divided by the total number of kernels; (2) S = total quantity of fungicide carried in excess by the R-kernels present, above the average for the N-kernels, divided by the amount of fungicide added in the process; (3) $T = S/R + 1$, which is the relative dose for the R-kernels. In general the group of R-kernels is of no practical importance, because it constitutes only a very small fraction of the treated seed. These distribution parameters, because of the limitations of the analytical methods refer to a kind of macrodistribution with respect to the world of the pathogens carried by the seed. However, the processes—e.g., vapor action and diffusion—which contribute to a uniform macrodistribution and are responsible for the penetration of the fruit coat may also produce a desirable microdistribution in the fruit coat.

Concept of Disease Center. The condition of the seed and the improvement given by seed treatment are considered in a schematic way. Without seed treatment the stand is reduced by the fraction of diseased plants X (number of diseased plants divided by the total number). Seed treatment disinfects the fraction Z of the diseased kernels, so that the fraction of healthy plants is then $(1 - X + XZ)$, the improvement given by seed treatment being $(X \times Z)$.

Actual values for X and Z are determined in laboratory, greenhouse, or field tests. Whether the seedling emerging from the embryo will grow to a healthy plant or become a victim of the microorganisms carried by the seed is dependent on a large number of factors. These factors are associated with the seed, the pest, the pesticide used in the treatment, the environment, etc. They are not discussed here. It is assumed that each kernel, which will result in a diseased seedling under the conditions of the actual test, contains at least one disease center (only seed-borne diseases are considered). These disease centers are assumed to be equivalent from the point of view of disease development and disinfection. Furthermore it is assumed that the disease centers are statistically distributed between the kernels of the seed.

A single spore is evidently the smallest possible disease center, but a disease center may also comprise an infestation of a number of spores and hyphae or aggregations of hyphae in a suitable location on the kernel.

Because the disease centers are assumed to be statistically distributed, some kernels may carry more than one disease center. Kernels carrying several disease centers should be more difficult to disinfect than those with only one. If no disinfection is performed, every kernel carrying one or several disease centers produces a diseased plant.

The definition of disease center is thus based directly on the emergence picture in the actual test. The concept of disease center is useful for the present purpose, because it enables us to discuss the disinfection process in general terms without considering the actual disease and the detailed mechanism of disinfection.

Distribution of Disease Centers. Application of fungicide on an infected kernel reduces the probability of disease development to a degree, which is dependent on the amount, n, of the fungicide. The probability that a disease center will become disinfected by the fungicide is written $\varphi(n)$; $[n = 0, \varphi(n) = 0; n = \infty, \varphi(n) = 1]$. The probability

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of disease development from this disease center is then $1 - \varphi(n)$. If a kernel is carrying h disease centers, the probability of complete disinfection is $[\varphi(n)]^h$ and the probability that at least one disease center will escape is accordingly $\{1 - [\varphi(n)]^h\}$. The disease centers are assumed to be distributed according to a Poisson distribution, because the number of disease centers in general is less or of the same order as the number of kernels. The frequencies of kernels carrying varying number of disease centers are then

Disease Centers per Kernel	Frequency
0 (healthy kernels)	e^{-m}
1 (diseased kernels)	$m \times e^{-m}/1!$
2 (diseased kernels)	$m^2 \times e^{-m}/2!$
3 (diseased kernels)	$m^3 \times e^{-m}/3!$
h (diseased kernels)	$m^h \times e^{-m}/h!$

Summation gives, remembering the definition of X,

$$X = \Sigma_1^\infty m^h \times e^{-m}/h! \quad (1)$$

$$1 - X = e^{-m} \quad (2)$$

After disinfection, the frequencies of not disinfected kernels (kernels with at least one remaining intact disease center) are obtained as

Disease Centers per Kernel Originally Present	Frequency of Kernels Not Disinfected
1	$\{1 - \varphi(n)\} \times m \times e^{-m}/1!$
2	$\{1 - [\varphi(n)]^2\} \times m^2 \times e^{-m}/2!$
3	$\{1 - [\varphi(n)]^3\} \times m^3 \times e^{-m}/3!$
h	$\{1 - [\varphi(n)]^h\} \times m^h \times e^{-m}/h!$

Summation gives

$$X - XZ = \Sigma_1^\infty \{1 - [\varphi(n)]^h\} \times m^h \times e^{-m}/h! \quad (3)$$

Introduction of the expressions derived in 1 and 2 gives

$$XZ = \Sigma_1^\infty [\varphi(n)]^h \times m^h \times e^{-m}/h! = e^{-m}\{e^{m \times \varphi(n)} - 1\} \quad (4)$$

$$XZ/(1 - X) = e^{m \times \varphi(n)} - 1 \quad (5)$$

$$\varphi(n) = \ln \frac{1 - X}{1 - X + XZ} \Big/ \ln (1 - X) \quad (6)$$

$$1 - \varphi(n) = \frac{\ln (1 - X + XZ)}{\ln (1 - X)} \quad (7)$$

Equations 6 and 7 relate the probability of disinfection of a disease center, $\varphi(n)$, with the experimentally determined quantities X, the fraction of diseased plants, and Z, the disinfection efficiency.

Kinetics of Disinfection

The next step is to determine how the assumed unit process of disinfection, characterized by $\varphi(n)$, is governed by the amount of fungicide, n, where n is reported in suitable units, in this case as the number of molecules of fungicide per kernel.

The disinfection process involves a number of physical processes as well as chemical reactions between essential sites in the microorganisms and the molecules of the fungicide. One of these processes is likely to control the ultimate rate of disinfection. The rate of disinfection (equal to rate of disappearance of disease centers) may then be written

$$dh/dt = -c \times n^v \times h \quad (8)$$

where h is the number of disease centers carried by the considered kernel, c is a constant and v stands for the number of fungicide molecules which take part in the rate determining step. The number of remaining disease centers at the time, h_t, is obtained by integration of Equation 8.

$$h_t = h_0 \times e^{-c \times n^v \times t} \quad (9)$$

The ratio between the number of disinfected disease centers at the time t, $h_0 - h_t$, and the initial number, h_0, can evidently be regarded as the probability, $\varphi(n)_t$, that one special disease center will become disinfected during the time t.

$$\varphi(n)_t = \frac{h_0 - h_t}{h_0} = 1 - e^{-c \times n^v \times t} \quad (10)$$

$$\frac{1}{1 - \varphi(n)_t} = e^{c \times n^v \times t} \quad (11)$$

$$\Phi = \ln \frac{1}{1 - \varphi(n)_t} = c \times n^v \times t \quad (12)$$

Plotting Φ defined in Equation 12 as a function of n in a logarithmic diagram would thus produce a straight line with the slope equal to the reaction order v.

$$^{10}\log \Phi = v \times {}^{10}\log n + {}^{10}\log c \times t \quad (13)$$

Equation 13 refers to a single kernel of seed or to in vitro tests where h_0 and h_t can be determined directly to give $\varphi(n)$ and Φ. When the result of the seed treatment is evaluated in terms of X and Z, as is generally the case, the function Φ is obtained from Equation 7 as

$$\Phi = \ln \frac{1}{1 - \varphi(n)} = \ln \frac{\ln (1 - X)}{\ln (1 - X + XZ)} \quad (14)$$

Subscript t on $\varphi(n)$ is dropped, because the influence of time is not considered here, only the influence of the fungicide concentration at constant time, t. Again plotting of Φ, evaluated according to Equation 14 on a logarithmic paper as a function of n should give a straight line as indicated in Equation 13.

Experimental Check of Theory

Frequently first-order kinetics, $v = 1$, is observed in disinfection reactions (5). This is illustrated by a typical example from the present field.

A useful method for testing seed disinfectants is the *Ustilago avenae* test. In this test oat seeds are artifically inoculated with spores of *Ustilago avenae*. The seed is treated with disinfectant and the spore germination determined microscopically on spores extracted from the seed. In this way h_0 and h_t are determined directly to give $\varphi(n)_t$ of Equation 10. (In this case a viable spore is considered a disease center.)

Figure 1 shows data obtained from *Ustilago avenae* tests on oats disinfected with various amounts of a cyano(methylmercuri)guanidine solution with 0.8% mercury and a dust of methoxyethylmercuric silicate with 1.5% mercury (3). First-order kinetics is obeyed in these two cases. The diagram shows that cyano(methylmercuri)guanidine is three times more efficient than methoxyethylmercuric silicate in this test, to produce the same value for Φ and $\varphi(n)$.

Seed treatment tests are in general performed only at two or sometimes three different rates and at high disinfection efficiencies, Z near 1. Data suitable for use in this connection are therefore scarce. Figure 2 contains three plots of

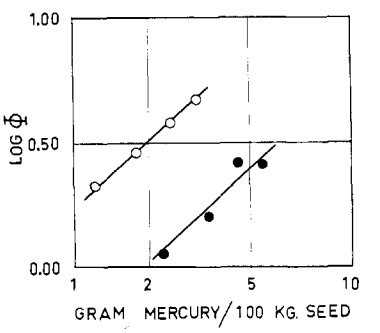

Figure 1. Data from Ustilago test plotted in a log Φ-log dose diagram according to Equation 13

● Treatment with methoxyethylmercuric silicate
○ Cyano (methylmercuri) guanidine (3)

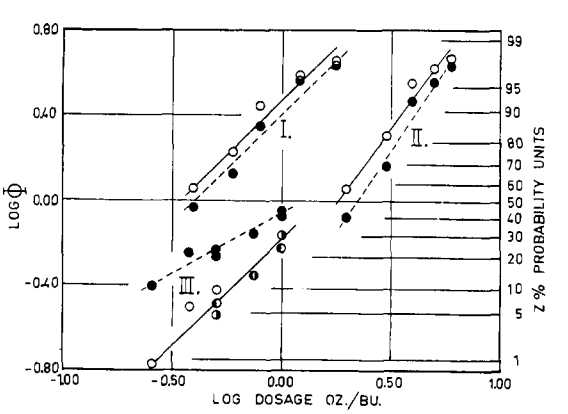

Figure 2. Results from seed treatment tests with log Φ as a function of log dose

I. Treatment of flax with Panogen 4930 (1.5% Hg) for control of *Alternaria*. $X = 0.86$ (3)
II. Treatment of wheat with Vancide 51 for control of bunt, $X = 0.92$. Average results used for 0, 1, 2, and 4 days' storage time before planting (2)
III. Treatment of oats with Parson's Seed Saver Dust (3.8% Hg) for control of smut, $X = 0.39$. Planted 1 day ◐; 1 week ◑; and 4 weeks ● after treatment (6)

available data. Reasonably linear relations are obtained in harmony with the theory.

The usual method of treating biological test results is to set up the familiar dosage-response curves (*1*, *4*). Percentage kill is plotted on a logarithmic-probability paper, which in general gives linear relations. Dosage-response curves are frequently used for in vitro tests. Introduction of standard deviation units (or probits) takes care of the variation in the sensitivity of the spore population toward the fungicide. The linear response to log dose is believed to be connected with the "logarithmic effect" frequently observed in biology and physiology. Application of the dosage-response curve technique on results from seed treatment tests (Z plotted on the probability axis) might thus be an alternative procedure. For comparison conventional dosage-response curves are also plotted in Figure 2. The two procedures give roughly the same type of curves and in practice it would be difficult to determine which approach gives the best linear fit of the experimental results. One advantage with the present approach is that it is performed in a straightforward manner on clear premises. Thus the slope of the curve is equal to the reaction order of the rate-determining step. This demonstrates clearly that the slope is directly associated with the disinfection mechanism.

Table I reports figures for the reaction order, v, calculated by Equation 13, for some seed treatment results published in the literature. These figures are based on only two rates, differing by a factor of 2 or less, and are accordingly not very exact. Nevertheless the data indicate that first-order kinetics, in general, may be applied also on results from field tests.

At high efficiencies, Z near 1, the slopes decrease in general. This is due, for example, to the fact that the disease centers are probably not equivalent in practice. It is believed that there are always present a small number of disease centers, which are particularly difficult to disinfect. Presence of these resistant disease centers will give decreasing slopes when Z is near 1. These effects are observed irrespective whether the material is treated by means of dosage-response curves or according to the present approach.

Table I. Apparent Reaction Order, v, Calculated from Field Test Results

Crop	Disease	X	Disinfectant	Dosages, Ounce/Bushel α	Dosages, Ounce/Bushel β	X − XZ α	X − XZ β	Reaction Order, v	Reference
Wheat	Bunt	0.92	Ceresan M	1/2	1	0.07	0.02	0.4	(2)
			Panogen	3/4	1 1/2	0.09	0.03	0.4	(2)
			Setrete	1/2	1	0.10	0.03	0.5	(2)
			5025-S	3/4	1 1/2	0.44	0.18	0.8	(2)
Oats	Smut	0.76	Agrox	1/2	1	0.34	0.07	1.3	(2)
			Mergamma	2	4	0.29	0.05	1.2	(2)
			N. I. Ceresan	1/2	1	0.21	0.04	1.0	(2)
Sorghum	Smut	0.286	MEMA	3/4	1 1/2	0.222	0.172	1.0	(7)
			Merculine	3/4	1 1/2	0.224	0.153	1.3	(7)
			Phygon	2	4	0.033	0.005	0.9	(7)
			Arasan	3	4	0.092	0.060	1.1	(7)

Table II. Drop in XZ, Δ × 100%, as a Function of Reaction Order v, and Spreading Error, σ_Q/Q

[Calculated by Equations 17 and 18 (Equation 18 figures given in parenthesis). Reference system $X = 0.10$ and $Z = 0.99$ for $\sigma_Q/Q = 0$]

Spreading Error, σ_Q/Q	Reaction Order, v 0.5	1.0	2.0	3.0
0	0	0	0	0
0.1	0.00 (0.00)	0.01 (0.01)	0.05 (0.04)	0.09 (0.09)
0.2	0.02 (0.01)	0.05 (0.05)	0.21 (0.18)	0.42 (0.39)
0.3	0.05 (0.03)	0.16 (0.11)	0.51 (0.44)	0.91 (0.96)
0.4	0.12 (0.07)	0.35 (0.23)	0.91 (0.88)	1.5 (1.8)
0.5	0.18 (0.11)	0.58 (0.42)	1.3 (1.5)	1.9 (2.8)

Calculations

The derived relation between disinfection efficiency and dose (Equations 13 and 14) will be used to estimate the difference in biological performance between a lot of treated seed with a completely uniform distribution, giving $(XZ)^0$, and a lot of seed characterized by definite values for the distribution parameters giving $(XZ)^1$. The drop in the fraction of recovered kernels denoted by Δ, is thus a direct measure of the influence of the distribution on the emergence picture.

$$\Delta = (XZ)^0 - (XZ)^1 \qquad (15)$$

The influence of the spreading error, σ_Q/Q, is considered first. The resulting $(XZ)^1$ is obtained by integration

$$(XZ)^1 = \int_0^\infty G(n) \times X \times Z(n) \times dn \qquad (16)$$

where $G(n) \times dn$ is the fraction of kernels with fungicide contents between n and $n + dn$ and $Z(n)$ governed by Equations 13 and 14. This integration is performed in an approximate, numerical manner with the smooth distribution curve substituted for a histogram with the staple width equal to $0.1 \times n_a$, n_a being the average amount of fungicide per kernel. The fraction of disinfected kernels $(XZ)^1$ is then obtained by summation

$$(XZ)^1 = \sum_1^{20} w_i \times (XZ)_i \qquad (17)$$

where w_i is the fraction of kernels with fungicide contents between $i \times 0.1 \times n_a \pm 0.05 \times n_a$ and $(XZ)_i$ is calculated for $i \times 0.1 \times n_a$.

This procedure is compared with a very rough estimation in which the lot of treated seed is considered to be composed of equal amounts of two populations with fungicide contents of $n(1 + \sigma_Q/Q)$ and $n(1 - \sigma_Q/Q)$, respectively.

$$(XZ)^1 = 0.5(XZ)_{n(1 + \sigma_Q/Q)} + 0.5\,(XZ)_{n(1 - \sigma_Q/Q)} \qquad (18)$$

The numerical values for the (XZ) terms in Equation 17 are calculated from Equation 13 with Φ as given by Equation 14. The constant term[10] $\log ct$ is calculated for reference systems with $Z = 0.99$, $X = 0.10$ and $v = 0.5$, 1, 2, and 3. Values for the difference Δ (Equation 16) have been calculated for various σ_Q/Q and are reported in Table II. This table also contains data which were arrived at by means of the very approximate Equation 18. Table II shows that the two procedures give similar results for moderate values of σ_Q/Q.

A practical conclusion from this agreement is that an N-kernel population characterized by σ_Q/Q can be treated with respect to disinfection efficiency as made up of two equally large populations with the doses $(1 + \sigma_Q/Q)$, and $(1 - \sigma_Q/Q)$ respectively, times the added average dose.

The difference, Δ, is roughly proportional to the square of the apparent reaction order, v. Accordingly, disinfection processes of higher order will be much more influenced by improper distribution. However, as shown above, the reaction order is in general about first-order.

At normal reaction orders $v \sim 1$, $\sigma_Q/Q \sim 0.2$ to 0.3 reduces the disinfection efficiency from $Z = 0.99$ in the reference system ($\sigma_Q/Q = 0$) with about

Table III. Drop in XZ, Δ × 100%, as a Function of Spreading Error, σ_Q/Q, for Various Reference Systems

(Z = 0.99, 0.90, and 0.50, X = 0.10 and 0.50 at σ_Q/Q = 0. Reaction order v = 1. Figures calculated by Equations 17 and 18. Equation 18 figures in parenthesis)

		Spreading Error, σ_Q/Q					
Z	X	0	0.1	0.2	0.3	0.4	0.5
0.99	0.10	0	0.01 (0.01)	0.05 (0.05)	0.16 (0.11)	0.35 (0.23)	0.58 (0.42)
	0.50	0	0.07 (0.06)	0.31 (0.26)	0.91 (0.64)	2.0 (1.3)	3.2 (2.4)
0.90	0.10	0	0.03 (0.02)	0.11 (0.11)	0.27 (0.25)	0.50 (0.46)	0.74 (0.75)
	0.50	0	0.16 (0.15)	0.65 (0.60)	1.5 (1.5)	2.7 (2.7)	3.9 (4.3)
0.50	0.10	0	0.01 (0.01)	0.05 (0.05)	0.11 (0.11)	0.18 (0.19)	0.27 (0.31)
	0.50	0	0.06 (0.05)	0.24 (0.25)	0.53 (0.55)	0.76 (0.90)	1.3 (1.5)

Table IV. Drop in XZ, Δ × 100%, as a Function of Reaction Order v and S-Factor

(Reference system X = 0.10, Z = 0.99, S = 0, and σ_Q/Q = 0)

	Reaction Order, v			
S	0.5	1	2	3
0	0	0	0	0
0.10	0.03	0.06	0.14	0.25
0.20	0.06	0.15	0.44	0.87
0.30	0.11	0.30	0.97	2.0
0.40	0.19	0.54	1.8	3.7
0.50	0.29	0.90	3.1	5.6

1%. It is, however, surprising to see that even for σ_Q/Q = 0.5 the drop Δ is not very large at least not when $v \leq 1$.

Table III shows how Δ is influenced by the chosen reference system. The ratio Δ/X is roughly the same for X = 0.10 and X = 0.50 in the studied cases, which indicates that the observed disinfection efficiency is only slightly influenced by X. On the other hand Δ varies in a complex manner with Z of the reference system with higher values reported for Z = 0.90 than for Z = 0.99 and Z = 0.50. The data in the table furthermore indicate that when Z = 0.50 (because of underdosage or less efficient fungicide) poor distribution is less detrimental than in case of an efficient treatment Z = 0.99. This is because in the former case the higher doses on some of the kernels will give a substantial gain in number of recovered kernels and thus partly compensate for the poor disinfection of the deficient kernels. In practice one is, however, interested only in efficient treatments with Z near unity.

The presence of the R-kernel population reduces the amount of fungicide to be shared between the N-kernels. The excess of fungicide on the R-kernels was characterized by the S-factor and the average dose on the N-kernels is then reduced to n_a^1 given by

$$n_a^1 = n_a(1 - S) \qquad (19)$$

The number of R-kernels is in general very small, $R \sim 0.02$, and the influence of this population on the observed disinfection efficiency is neglected.

Table IV gives some data on the drop in the fraction of recovered kernels due to various S-factors, calculated by means of Equations 19, 15, 13, and 14. At normal reaction orders, $v \sim 1$ values for the S-factor around S = 0.1 to 0.2 reduces the disinfection efficiency from Z = 0.99 in the reference system with about 1%.

Discussion

Radioactive distribution studies reported earlier (*9*) have shown that distribution parameters for treated seeds (wheat) under normal conditions in case of liquid seed treatment (Panogen process) amount to $\sigma_Q/Q \sim 0.2$ to 0.3 and $S \sim 0.05$. According to the reported numerical estimates, the drop in disinfection efficiency associated with this distribution pattern is expected to be only a few per cent, comparison made with a hypothetical distribution σ_Q/Q = 0 and S = 0. This is satisfactory and further improvement of the distribution, if possible, would be of no practical value—e.g., reduction of disinfection efficiency with 2% in case of a lot of seed with 10% diseased kernels (X = 0.10) would reduce the healthy stand from 99.9 to 99.7% if the disinfection efficiency Z is assumed to be Z = 0.99 in the ideal case.

A distribution which is completely homogeneous in a mathematical sense may, however, never be obtained in practice, because of the differences between individual kernels of seed not only with respect to size, but also with regard to the factors governing the resorption of the fungicide. This was demonstrated clearly in an earlier distribution study with a mixture of mechanically damaged and intact kernels (Table XV, *9*). In this case the spreading error σ_Q/Q actually increased with increased storage time, because of the widely differing properties of the individual kernels of seed.

The calculations performed were based on the assumption of a normal distribution of the fungicide contents of the treated kernels. However, actual distribution curves in general exhibit fewer deficient kernels than required by a strictly normal distribution. Estimates of Δ are therefore conservative. The only requirement on the used relations from this point of view is that they should satisfy the experimental data reasonably well, which obviously is the case.

A further check of the theory than given in this paper (cf. Figure 2) might evidently be obtained by plotting the function Φ *vs.* time t in a logarithmic diagram which should also give a straight line according to Equation 13. Unfortunately, time t cannot be evaluated in these biological tests. When the seed is planted immediately after the treatment, disinfection will still proceed for a certain time after the planting and the time t to be used in the kinetic expressions can thus not be determined with sufficient confidence. On the other hand, in case of a long storage time before planting, the time available for the disinfection reactions will be less than the actual storage time, because of the influence of competing reactions in the surface layer of the seed kernel. Reaction kinetics is therefore preferably evaluated by analysis of tests at constant time t but with varied amounts of added fungicide.

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