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Measures of disease intensity in powdery mildew (Erysiphe graminis) of winter wheat. 2. Relationships and errors of estimation of pustule number, incidence and severity

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Abstract

Assessments of pustule number, incidence and severity of powdery mildew on winter wheat in the Netherlands were made in commercial fields and in experimental plots. Assuming a constant leaf and pustule size, a leaf can carry at most M_m pustules. If the number of clusters, each consisting of n pustules, follows a binomial distribution, then the relation between incidence (I, proportion diseased leaves) and severity (S, proportion diseased leaf surface) is: $(1 - S) = (1 - I)^{n/M_m}$. The model explains the main effects of leaf, pustule and cluster size on incidence-severity relations and gave a good description of the measured relation. The negative binomial distribution poorly described the measured relation. The model of Nachman (1981) gave a good description of the relation between mean pustule number ($\overline{m}$) and incidence (I). The relation found in commercial fields, irrespective of fungicide treatments, was: $\ln(\overline{m}) = 1.48 + 1.14 \ln(\ln[1/(1 - I)])$. The effects of years, varieties, growth stages and leaf positions on this relation were not significant. Incidence assessments can be used to predict the pustule number, but this method is less efficient than the use of direct pustule counts. Estimates are given of the variance of the predicted pustule number.

Additional keywords: Triticum aestivum, epidemiology, sampling method, detection level, multiple infection.

Introduction

In a previous paper the precision of counting pustules as a method to assess mildew *(Erysiphe graminis)* intensities in field experiments was evaluated. Pustule counts can be used easily by a farmer to monitor crops with low mildew intensities, but this method cannot be used for all the diseases he may need to monitor. Recently, incidence assessments (proportion of leaves with disease) have been used in disease and pest monitoring by farmers (Rabbinge and Mantel, 1981; Rijsdijk, 1982; Zadoks, 1984). Incidence assessment is simple, and, since the same method can be used for different diseases and pests, it might facilitate the adoption of supervised control systems. Seem (1984), reviewing disease incidence-severity relations, pointed out that no consensus existed on the various assessment methods. He proposed that severity should be defined as 'the quantity of disease affecting entities within a sampling unit'. This is a very broad definition including both absolute and relative measures. However such a distinction

is needed in this paper. Following Seem (1984), with modifications, the following terms will be used:
intensity is used to refer to the quantity of disease, irrespective of the assessment method;
pustule number is the disease intensity expressed as the number of pustules on a leaf, so it can be considered as a relative density;
severity is the disease intensity expressed as the proportion of leaf surface which is visibly diseased;
incidence is the disease intensity expressed as the proportion of leaves which are visibly diseased.

James and Shih (1973) studied the disease incidence-severity relation for powdery mildew in commercial wheat fields. They concluded that incidence can be used to estimate severity at low disease intensities. Rouse et al. (1981) studied the incidence-severity relation for the same disease in experimental plots. They used the model of James and Shih (1973), but found different parameter values. Ward et al. (1985a, b) studied the precision of two sampling methods for cereal aphids and concluded that incidence sampling was not significantly less accurate than direct counting.

The objective of this paper is to analyse and describe the incidence-severity relation for mildew in the Netherlands. The precision of this relation when used as a calibration line in supervised control systems is evaluated and compared to the precision of direct pustule counts.

Materials and methods

Data. This paper is based on two data sets, one from commercial fields and one from field experiments, both in the Netherlands. A description of the assessment methods and statistics used is given by Daamen (1986).

Sample variance of incidence. Let D be the number of diseased leaves in a random sample of size n, from a field in which a proportion p of the leaves is diseased. Then D will follow a binomial distribution with parameters p and n. The sample variance of the proportion of leaves with disease (I) is given by:

$$\mathrm{Var}\ (\mathrm{I}) = \frac{p \cdot (1 - p)}{n} \tag{1}$$

In contrast to the direct or pustule counts, the sample variance of incidence is independent of the disease species. Statistical tables are available for confidence limits of proportions (Rohlf and Sokal, 1969).

The detection level of the sampling method depends only on the sample size. The detection level is defined as that disease incidence (p_d) at which there is a 97.5% chance of finding the disease in a sample of n leaves:

$$(1 - p_d)^n = 0.025 \tag{2}$$

Solving (2) gives for $n = 100$ a detection level $p_d = 0.036$; and with $n = 500$, $p_d = 0.0074$. Thus sampling 100 leaves from a field in which 3.6% of the leaves are diseased results in a probability of 2.5% that the sample is clean.

Incidence-severity models. It is reasonable to assume that spores from a cloud landing on a crop are randomly distributed over the leaves. If each landed spore causes disease, then the relation between the mean incidence (I) and the mean pustule number M can be described using the zero term of the Poisson distribution (multiple infection transformation, see Gregory, 1948):

$$(1 - I) = e^{-M} \tag{3}$$

If pustule and leaf size are constant, and a leaf can carry a maximum of M_m pustules, the relation between pustule number and severity (S, proportion leaf surface diseased) will be:

$$S = \frac{M}{M_m} \tag{4}$$

Substitution of (4) into (3) yields the incidence-severity relation:

$$(1 - I) = e^{-M_m \cdot S} \tag{5}$$

Spore dispersion is random only at the start of an epidemic in a field. Normally clustering will occur, owing e.g. to the failure of spores generated by a pustule to escape from a leaf. Assume that clustering results in clusters with a constant number, n, of pustules. The mean number of clusters on a leaf, N, equals M/n. Then a random distribution of clusters on the leaves following a Poisson distribution will give the incidence-severity relation:

$$(1 - I) = e^{-N};\ N = \frac{M}{n} \tag{6}$$

Elimination of N gives:

$$(1 - I) = e^{-\frac{M}{n}}\ \text{, or}\ \ (1 - I) = e^{-\frac{M_m}{n} \cdot S} \tag{7}$$

Model 7 is in fact equivalent to (5), it merely considers clusters of n pustules rather than single pustules with a Poisson distribution.

The assumptions of a Poisson distribution are not violated statistically when disease intensity is low. At higher disease intensities the probability that two or more cluster-initiating spores land in the same place and form only one visible cluster cannot be neglected. Moreover, a leaf cannot carry more than N_m clusters ($N_m = M_m/n$). If A cluster-initiating spores land at random on a leaf, each potential cluster site receives, on average, A/N_m cluster-initiating spores. The resulting mean number of visible clusters (A_v) on a leaf will be (Poisson distribution, see also Justesen and Tammes, 1960):

$$A_v = N_m(1 - e^{-\frac{A}{N_m}}), \text{ or } \quad e^{-A} = (1 - \frac{A_v}{N_m})^{N_m} \tag{8}$$

in which $A_v = M/n$; M is the number of visible pustules per leaf; and $N_m = M_m/n$. Substitution of (8) into (6) yields:

$$(1 - \frac{A_v}{N_m})^{N_m} = (1 - I)\ ; \quad A_v = \frac{M}{n}, \quad N_m = \frac{M_m}{n}$$

Elimination of A_v and N_m and substitution of (4) yields the incidence-severity relation:

$$(1 - S) = (1 - I)^{\frac{n}{M_m}} \tag{9}$$

where S and I are the severity and incidence in proportions, n is the cluster size (in pustules) and M_m is the maximal number of pustules on a leaf.

Model 9 can also be derived in one step, assuming that a leaf can carry a = 0, 1, 2....N_m clusters of n pustules ($N_m = M_m/n$). If the number of clusters per leaf follows a binomial distribution, the probability of sampling a leaf without clusters will be:

$$p(a = 0) = (1 - \frac{A_v}{N_m})^{N_m}\ ; \quad N_m = \frac{M_m}{n}, \quad A_v = \frac{M}{n} \tag{10}$$

and (10) can be rewritten as incidence-severity relation 9, because $p(a = 0) = (1 - I)$ and $M/M_m = S$ (4). A Poisson distribution will approach the binomial distribu-

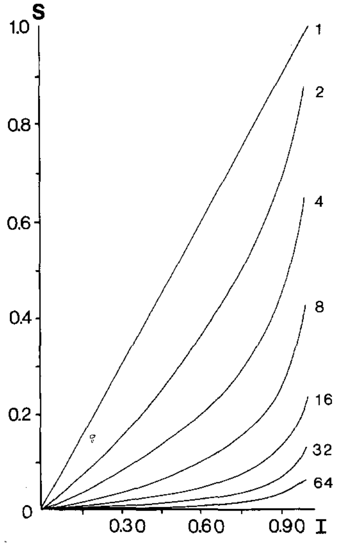

Fig. 1. Incidence (I) and severity (S) relations generated by the binomial model (see text). The data in the figure represent the maximal number of clusters per leaf.

tion when N_m is large and A_v is small, then (7) may be considered as a special case of (9).

Incidence-severity relations, generated by the binomial model (9) are shown in Fig. 1. All the generated incidence-severity relations pass through the origin. The relation simplifies to the straight line $S = I$, if one cluster destroys the whole leaf ($n = M_m$). When all leaves in an infinitely large population are diseased; $I = 1$ with solution $S = 1$. Since n/M_m is usually much smaller than unity, the slope of S is very steep near $I = 1$ (Fig. 1). For example when a leaf can carry 10 clusters of size n ($n/M_m = 0.1$) and 98% of the leaves are diseased ($I = 0.98$), then S is estimated as 0.32, or roughly one third of the leaf surface is diseased.

James and Shih (1973) proposed the exponential incidence-severity relation:

$$I = (1 - r^{100 \cdot S})\;; \quad 0 < r < 1 \tag{11}$$

in which parameter r defines the slope of the exponential relation and S represents the severity as a proportion, which equals M/M_m (4). Thus (11) can be rewritten:

$$(1 - I) = e^{100 \frac{M}{M_m} \cdot \ln(r)}\;; \quad 0 < r < 1 \tag{12}$$

and becomes (7) when:

$$\frac{M_m}{n} = -100 \cdot \ln(r), \text{ or } \quad r = e^{-\frac{M_m}{100 \cdot n}} \tag{13}$$

Models 7 and 11 are thus equivalent; they differ only in their underlying assumptions and the meaning of the parameters. James and Shih (1973) do not explicitely state the underlaying assumptions of the model. The parameter, r, of their model is difficult to interpret, but it can be calculated (13) from the parameters cluster size (n) and the maximal number of pustules on a leaf (M_m), assuming mildew clusters follow a Poisson distribution. Since model 7 is a special case of (9), so is model (11).

Nachman (1981) proposed the following relation between incidence and mite density:

$$(1 - I) = e^{-t \cdot M^u} \tag{14}$$

in which t and u are parameters and M is the mean density, or, in the context of mildew infestations, the mean pustule number. Nachman (1981) derived the model without explicit assumptions about any statistical distribution. Comparison of (14) and (7), shows that (7) is a special case of (14). If parameter u of equation 14 equals unity, the models are equal and the parameter n (cluster size) is then the inverse of parameter t of the Nachman model.

Waggoner and Rich (1981) concluded that the distributions of diseases they studied usually followed the negative binomial distribution. Their incidence-severity relations were generally also described by the first term of the negative binomial:

$$(1 - I) = \left(\frac{M}{k} + 1\right)^{-k} \tag{15}$$

where k is a positive parameter indicating the degree of clustering. Comparison of models 7, 9 and 14 with model 15, do not reveal any condition under which the models are equal. If parameter k of (15) approaches infinity, (15) becomes (3), but that is not interesting.

Results

Estimation of parameters for the incidence-severity relations. The binomial incidence-severity relation (9), the Nachman model (14) and the negative binomial (15) were fitted to the data. Since the data are taken from samples of the unknown disease incidence and severity in the field, both variables are measured with error. Here, we consider the data now as exact measurements (at least the incidence on the samples); the error of estimation will be treated in the next section. Taking logs, model 9 is rewritten:

$$\ln\left(\frac{1}{1-S}\right) = \frac{n}{M_m} \cdot \ln\left(\frac{1}{1-I}\right) \tag{16}$$

The ratio n/M_m is treated as one parameter: q. The model has a test condition; the described relation must pass through the origin when fitted to the data. Inspection of residuals revealed heteroscedasticity; by trial and error a fourth root transformation was found to stabilize the variances. Analyses of variance were carried out on the data sets of commercial fields and experimental plots to test for deviation of the intercept from zero (test condition) and for variation in q among years, varieties and growth stages (Table 1). For both data sets it can be assumed that q is constant: neither test revealed a significant difference. For the data set of experimental plots, however, the

Table 1. Analyses of variance to test for dependence of the incidence severity relation: $\ln(1 - S) = q * \ln(1 - I)$, in years, growth stage (DC $\leqslant$ 59; DC > 59) and varieties (Arminda, Okapi, Caribo). Commercial fields n = 42, years 1980, '81; experimental plots n = 32, years 1980 to 1983. Analysis of the transformed model, see text.

Source of variation	Comercial fields			Experimental plots		
	df	SS	F	df	SS	F
1. common q	1	2.900		1	2.286	
2. intercept	1	0.002	1.3ns[2]	1	0.022	15.6* * *[2]
3. residual	40	0.050		30	0.045	
4. different q	4	0.006	1.2ns			
4a. years	1	0.004	3.1ns			
4b. growth stage	1	0.000	0.0ns			
4c. varieties	2	0.002	0.6ns			
5. residual	36	0.044				
6. total[1]	42	2.952		32	2.352	

[1] Not corrected for the mean.

[2] ns = not significant ($p > 0.05$); * * * = significant at $p = 0.001$.

Table 2. Fitted values for the parameters of the incidence severity relation in the transformed model: $\ln[1/(1 - S)] = q \cdot \ln[1/(1 - I)]$, see text; their standard deviations; and estimates of q and its 95% confidence limits.

Location	df	q* *0.25	R^2	q
commercial fields	41	0.305 (0.006)	0.98	0.008 (1.010-0.007)
experimental plots	31	0.312 (0.009)	0.97	0.009 (0.012-0.007)

relation does not pass through the origin. The intercept is estimated as +0.11 (s.e.: 0.03). After retransformation the incidence-severity relation does not pass through the origin, as the severity is 0.00017 (= 0.017 percent) at zero incidence. Though the deviation is statistically significant, it is negligibly small from a biological point of view. The disease intensities in the experimental plots covered a greater range than those in the commercial fields, where fungicides were used to control severe outbreaks of mildew. Because of this greater range, a significant deviation of the intercept from zero can be detected more easily.

When the equation is forced through the origin, the estimates of parameter q (n/M_m) do not differ significantly for the two data sets (see Table 2). The R^2 values are high, so the transformed model gives a good description of the data. As M_m was 500 (see Methods), estimates of the average cluster size (n) are: 4 for the commercial fields and 4.5 for the experimental plots. It is interesting to compare the estimates of q with the estimates of parameter r in Canada (James and Shih, 1973). The estimates of parameter r ranged from 0.30 to 0.67 for individual leaf positions. These values correspond with values of q ranging from 0.008 to 0.025 (13). The value estimated here of 0.009 for the three top leaves is within this range. Another comparison can be made with the data of Rouse et al. (1981), who used the model of James and Shih (11) to describe the relation for a field experiment in the USA. They estimated a parameter value which corresponds to a very large value of q: 0.076 (see Discussion). Zaharieva et al. (1984) described incidence-severity relations for mildew on winter wheat in Bulgaria. The parameters they estimated can not be compared with the estimates of q, as they used other models to describe the incidence-severity relation.

The more complex Nachman model (14) was also fitted. Taking logs and redefining the parameters, the model was rewritten:

$$\ln(\overline{m}) = a + b \cdot \ln\left[\ln\left(\frac{1}{1 - I}\right)\right] \qquad (17)$$

This model is linear with the parameters $a = (1/u) \cdot \ln(1/t)$ and $b = 1/u$. Analyses of variance were carried out to test for deviations due to years, varieties or growth stages from common parameters a and b, see Table 3. Residuals showed no heteroscedasticity. The results of these tests showed no significant deviations due to years, varieties or growth stages. The estimates of the parameters are given in Table 4. An estimate of 1/t of the original model (14) is given, because this is an estimate of the average cluster size if u equals 1, as discussed above. For the experimental plots the parameters were also estimated for the individual leaf positions. No significant effects of leaf posi-

Table 3. Analyses of variance to test for dependence of the relation between incidence and mean pustule number: $\ln(\bar{m}) = a + b\cdot\ln(\ln[1/(1 - I)])$, on years, growth stage ($DC \leqslant 59$; $DC > 59$) and varieties (Arminda, Okapi, Caribo). Commercial fields n = 42, years 1980, '81. Experimental plots n = 32, years 1980 to 1983.

Source of variation	Commercial fields			Experimental plots		
	df	SS	F	df	SS	F
1. common a and b	1	74.5	240.7∗∗∗[1]	1	74.4	271.9∗∗∗
2. residual	40	12.4		30	8.2	
3. different a and b	8	3.3	1.5ns[1]	12	4.6	1.9ns
3a. years	2	1.4	2.5ns	6	2.0ns	
3b. growth stage	2	0.4	0.7ns	2	0.2	0.4ns
3c. varieties	4	0.9	0.8ns	4	1.0	1.2ns
4. residual	32	9.1		18	3.6	
5. total	41	86.8		31	82.6	

[1] ns = not significant; ∗∗∗ = significant at $p = 0.001$.

Table 4. Estimated parameters of the relation between incidence (I) and mean pustule number ($\bar{m}$) in the Nachman model: $\ln(\bar{m}) = a + b\cdot\ln(\ln[1/(1 - I)])$, their standard deviations; and an estimate of $1/t$, see text.

Leaf position	df	a	b	R^2	1/t
three top leaves					
commercial fields	40	1.48 (0.11)	1.14 (0.07)	0.86	3.6
experimental plots	30	1.63 (0.12)	1.42 (0.09)	0.90	3.2
single leaf layers experimental plots					
top leaf	30	0.89 (0.10)	1.19 (0.03)	0.98	2.1
2nd leaf	30	0.90 (0.08)	1.23 (0.05)	0.95	2.1
3rd leaf	26	1.07 (0.07)	1.37 (0.06)	0.95	2.2
average	88	0.99 (0.05)	1.24 (0.02)	0.97	2.2

tion on estimated parameters were found, so the estimates of common parameters a and b are also given. As an illustration a plot of data from the commercial fields is given (Fig. 2). The R^2 values are high, so the transformed model gives a good description of the data.

For the three top leaves together, estimates of parameter b from the experimental plots and from the commercial fields differ significantly. A small value of b indicates that pustule number increases less with increasing incidence than is the case with a larger value of b. The difference in estimates of b is in agreement with the difference in the measurement of incidence: on both surfaces of the leaves in the commercial fields and on the upper surface only in the experimental plots. For the commercial fields, the estimate of b is close to unity, and then models 9 and 14 are nearly the same. For this reason the conditions required to fit model 9 were satisfied in the commercial fields but not in the experimantal plots.

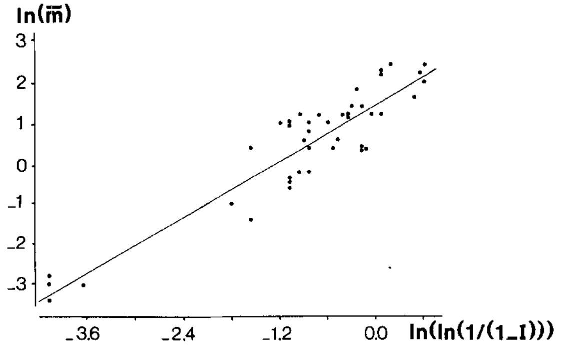

Fig. 2. Relation between transformed incidence ($\ln(\ln[1/(1 - I)])$) and log mean pustule number ($\ln(\overline{m})$); data from commercial fields, top three leaves together. Each point represents a sample of 40 tillers.
Regression: $\ln(\overline{m}) = 1.48 + 1.14 \ln(\ln[1/(1 - I)])$; $R^2 = 0.86$.

Comparison of the three top leaves together with the single leaf positions (experimental plots) shows that the R^2 values for the single leaf positions are slightly higher (Table 4). Both parameters, a and b, are larger for the three top leaves together than for the single leaf positions, resulting in a larger pustule number at equal incidences. This is a common result when averages are taken from non-linear relations (here a convex relation). As disease intensities on the different leaf positions are always different, the effect is fairly strong.

The estimates of the average cluster size (1/t) are 3.6 for the commercial fields and 3.2 for the experimental plots. These estimates are slightly lower than those obtained using the binomial model (4.5 and 4 respectively), because parameter b does not equal unity.

For the samples from the commercial fields, the fitted relation between incidence (I, proportion diseased leaves, n = 120, top three leaves both sides) and pustule number $\overline{m}$, average pustule number per leaf (upper surface) is:

$$\ln(\overline{m}) = 1.48 + 1.14 \ln\left[\ln\left(\frac{1}{1 - I}\right)\right] \qquad (18)$$

The relation between incidence and mean pustule number based on the negative binomial (15) could not be made linear in k and I. The model was fitted to the data of the experimental plots by an optimization procedure using least squares. The estimates of parameter k were 0.69 for the three top leaves together and 1.9, 0.68 and 0.57 for the top, second and third leaf, respectively. The fits were not acceptable. Moreover the Nachman model gives the same relation for each leaf position, while the negative binomial does not. An optimization assuming Poisson-distributed errors was also tried, but performed no better.

Precision of predicted pustule number from incidence and sample size. Both the binomial model and the Nachman model gave a good description of the measured incidence-severity relation. As the Nachman model is more flexible than the binomial model, the precision of the estimated pustule number from a new incidence count will be analysed using the Nachman model as calibration line. The relation between pustule number and incidence has been described with the assumption that incidence was measured without error (18). The variance of the predicted pustule number in a sample can be estimated as usual by:

$$\mathrm{Var}\,[\ln(\overline{m})] = \mathrm{MS_e}\cdot[1 + \frac{1}{n_c} + \frac{(x - x_c)^2}{\mathrm{SS_x}}] \qquad (19)$$

in which $\mathrm{MS_e}$ is the residual variance (= 0.31); n_c is the number of observations of the estimating set (= 42); x is the new observation (= ln [ln (1/(1 − I))]) made to estimate the pustule number; x_c is the mean value of x in the estimating set (= −0.855); and $\mathrm{SS_x}$ is the sum of squares of the x-values (= 57.2). Thus, for a new sample an estimate of the pustule number and its variance can be given, if incidence is measured as was done for the determination of the estimating set. However, we require an estimate of the pustule number in the field and of the dependence of its variance on the incidence count, when the latter is considered a random variable.

Equation 18 describes the conditional expectation of log (pustule number), given the incidence; and (19) describes its conditional variance. If we consider the incidence I as a random variable rather than a known fixed variable, the expectation of ln(M) can be estimated using (18). The variance of this estimate is more complex but it can be decomposed into two parts: the mean conditional variance and the variance of conditional mean (Rao, 1965):

$$\mathrm{Var}\,(\ln(\hat{M})) = \mathrm{E}\cdot\mathrm{Var}\,(\ln(\hat{M})\,|\,x) + \mathrm{Var}\,(\mathrm{E}\,(\ln(\hat{M})\,|\,x))$$

The mean conditional variance can be calculated from (19); the expectation of $(x - x_c)^2$ is equal to $(\mathrm{E}x - x_c)^2 + \mathrm{Var}(x)$. The variance of the conditional mean may be estimated by a Taylor approximation with respect to x (18). The 'unconditional' variance can thus be estimated as:

$$\mathrm{Var}[\ln(\hat{M})] \approx \mathrm{MS_e}\cdot(1 + \frac{1}{n_c} + [\frac{(\mathrm{E}x - x_c)^2 + \mathrm{Var}(x)}{\mathrm{SS_x}}]) + 1.14^2\cdot\mathrm{Var}(x) \qquad (20)$$

in which Ex can be estimated as ln(ln[1/(1 − I)]). The variance of x can be approximated by a Taylor expansion with respect to I:

$$\mathrm{Var}(x) = \frac{\mathrm{Var}(I)}{[\ln(1 - I)]^2\,.\,(1 - I)^2} \qquad (21)$$

The variance [Var(I)] of the new incidence count can be estimated from (1), so, from a new incidence count the pustule number in a field can be estimated by (18) and its variance estimated using equations 20, 21 and 1. If the incidence in a field is known accurately, (20) equals (19), so the variance of the estimated pustule number cannot be smaller than the conditional variance, under the assumption that incidence is not a random variable.

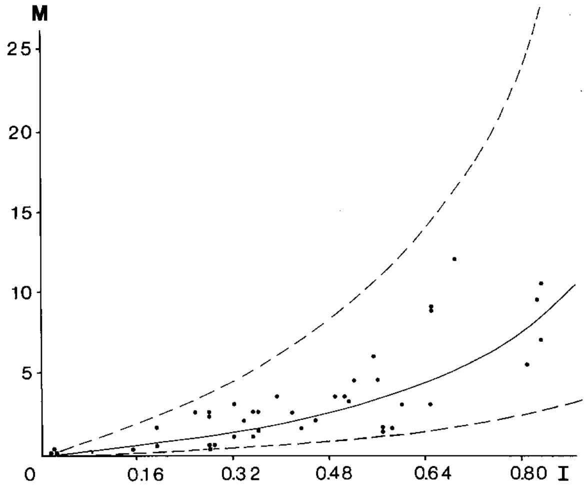

Fig. 3. Relation between incidence (I) and the mean (M) (solid line) and 95% confidence limits (broken lines) of the number of powdery mildew pustules; calculated using the Nachman model. Data from commercial fields.

Using the equations, 95% confidence limits were constructed and transformed to the original scales I and M. In Fig. 3 the relation between incidence and pustule number is plotted, with the data from the estimating set. Table 5 shows the confidence limits for the estimates of pustule number in samples and in fields, for different sample sizes. Clearly, the confidence limits are determined mainly by the conditional variance (19).

Table 5. Mean pustule number on upper surface of a leaf (M) estimated from the incidence (I), and estimated 95% lower (LL) and upper (UL) confidence limits for samples and three different sample sizes (n) for fields.

I	M	Samples (n = 120)		Fields (n = 60)		Fields (n = 120)		Fields (n = 240)	
		LL	UL	LL	UL	LL	UL	LL	UL
0.05	0.15	0.05	0.47	0.03	0.86	0.03	0.65	0.04	0.56
0.15	0.55	0.18	1.7	0.14	2.2	0.16	1.9	0.17	1.8
0.25	1.06	0.35	3.2	0.30	3.8	0.32	3.5	0.34	3.4
0.35	1.68	0.55	5.1	0.49	5.8	0.52	5.4	0.54	5.3
0.45	2.45	0.80	7.5	0.73	8.2	0.77	7.8	0.79	7.6
0.55	3.40	1.1	10	1.0	11	1.0	11	1.1	11
0.65	4.65	1.5	14	1.4	15	1.5	15	1.5	15
0.75	6.39	2.1	20	1.9	21	2.0	20	2.0	20
0.85	9.14	2.9	28	2.7	30	2.9	29	2.9	29
0.95	15.39	4.9	48	4.8	53	4.7	50	4.8	49

Efficiency of direct pustule counts and incidence counts as methods to estimate pustule number. To evaluate the efficiencies of direct pustule counts and incidence counts to predict the pustule number in a field, it is assumed that the duration to select a leaf from a field and the duration to count a pustule or to score a diseased leaf are equal and require t time units. Both methods are assumed to be equal in precision, if the 95% lower and upper confidence limits of the estimated pustule number are equal. The ratio of time units, both methods require at equal precision is a measure of efficiency of one method as compared to the other. It is not possible to calculate these ratios by means of a simple equation. The number of time units (t_i) needed to assess mildew incidence in samples of n_i (60, 120 and 240) leaves, at different mildew incidences (I) was calculated by:

$$t_i = n_i + n_i * I \tag{22}$$

The upper (UL) and lower (LL) 95% confidence limits of Table 5 were used to calculate the mean pustule number ($\overline{m}$) and the number of leaves (n_d), at which the direct pustule counts would give the same confidence limits. The 95% confidence limits for the direct pustule counts can be estimated by (Daamen, 1986):

$$UL = \overline{m} + 1.96\sqrt{\frac{2.2\,\overline{m}^{1.55}}{n_d}}, \text{ and } LL = \overline{m} - 1.96\sqrt{\frac{2.2\,\overline{m}^{1.55}}{n_d}} \text{ and} \tag{23}$$

$$\overline{m} = (UL + LL)/2, \text{ so} \tag{24}$$

$$n_d = \frac{2.2[(UL + LL)/2]^{1.55}}{[(UL - LL)/(3.92)]^2} \tag{25}$$

and the number of time units (t_d) needed for the direct pustule counts was computed by:

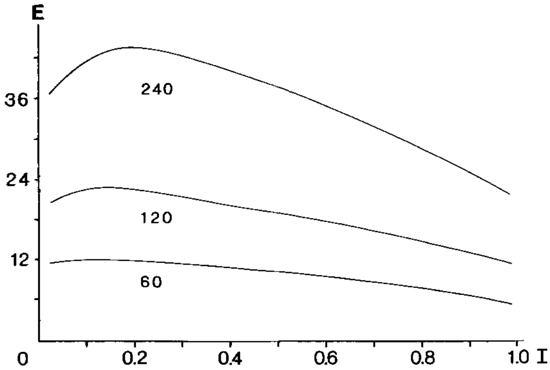

Fig. 4. Efficiency (E) of direct pustule counts compared to incidence counts, in relation to mildew incidence (I). The data in the figure represent the sample size (n = number of leaves) of the incidence counts.

$$t_d = n_d + n_d \cdot \overline{m} \tag{26}$$

The efficiency (E) of the direct pustule counts as compared to the incidence counts was computed by:

$$E = t_i / t_d \tag{27}$$

The estimated efficiencies, assuming that equal time units are required to sample a leaf or to score a diseased leaf or to score a pustule, are shown in Fig. 4. The sample size of the direct pustule counts is not fixed and can be computed from (25). It is clear that direct pustule counts are 10 to 20 times more efficient than incidence counts, when the sample size of the latter are 60 or 120 leaves.

Discussion

In this paper an incidence-severity relation was derived, assuming: constant leaf, pustule and cluster size; and a binomial distribution of the number of clusters on leaves. Although it is a crude simplification of the problem, it gives an explanation of effects of leaf, pustule and cluster size on incidence-severity relations. The model of James and Shih (1973) is a special case of the binomial model presented here. The underlying assumptions of the model of James and Shih have now been made explicit, and an equation has been given to calculate the parameter r of their model from the maximal number of clusters that a leaf can carry. The present model was developed for powdery mildew on fully grown winter wheat, where pustule and leaf size are fairly constant and cluster formation takes place by new infections. The model might also be applicable to analyse incidence-severity relations for diseases which exhibit a strong systemic growth in the leaf, when a minimum lesion size is defined. The cluster size then represents the average lesion size relative to the minimum lesion size.

James and Shih (1973) estimated the incidence-severity relation in Canada for mildew in commercial winter wheat fields, after the second node stage. The relation estimated in the Netherlands agrees with theirs; the distributions of mildew in the crops are roughly similar in the two continents. James and Shih (1973) concluded that the relation varied between years and depended on leaf position. The effects of these factors were not significant in the present study. For mildew in an experimental wheat field in the USA, before the second node stage, Rouse et al. (1981) found a different relation. They concluded that the early onset of the epidemic had caused this difference. In the context of the model developed in this paper, it is more plausible to explain the difference by the smaller leaf size of the young plants they studied as compared to the fully grown plants in this study. The effect of leaf size (as the maximal number of pustules a leaf can carry) on the relation is described by the model developed in this paper. Incidence-severity relations can be improved by taking leaf size into account. A problem to be solved is the relation between cluster size (or lesion size) and the state of the epidemic. In the present study, no attempt was made to expand the model to incorporate the age distribution of clusters or lesions.

The model of Nachman (1981) was used as a description of the relation between incidence and pustule number, and as a calibration line. No significant effects were found for different years, varieties, growth stages or leaf position. Since incidence and pustule number were not measured independently, Mandel's (1984) sophisticated method to remove bias and to estimate the error structure of the relation could not be used. Instead, conditional statistics were used to approximate the error in the pustule number in a field predicted from an incidence count of a sample. The precision with which the incidence is determined has little effect on the error of the estimated pustule number, which is relatively large and depends mainly on the conditional variance (19). The conditional variance depends mainly on the residual error of the fitted regression line. Although the residual error was relatively small (high R^2 values), it becomes relatively large after retransformation to the original scales, the error stands in the exponent. If it takes as long to count one diseased leaf (incidence) as to count one pustule, than the direct pustule count is far more efficient. Ward et al. (1985a, b) concluded that both methods, incidence and direct counting, were equal in precision over a range of aphid intensities, but they considered only the variance of the conditional mean. In the present study with powdery mildew, the mean conditional variance could not be neglected, since it was the main determinant of the error in the predicted pustule number. For powdery mildew in winter wheat, incidence counts have advantages above pustule counts for application in supervised control systems, but the disadvantage is that incidence counts are less efficient than direct pustule counts. From a research point of view, pustule counting is prefereable above incidence counting. From a farmers point of view, incidence counting is preferable to pustule counting or severity assessment as the method is applicable to all the diseases and pests he has to monitor in his wheat crop. Moreover, not only is the application easy, but also the traning of farmers. For these reasons in the Netherlands equation 17 plus one standard error is used in the EPIPRE system to transform farmers' incidence counts to pustule numbers since 1985.

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Samenvatting

Meting van meeldauwaantastingen (Erysiphe graminis) in wintertarwe. 2. Samenhang en schattingsfouten van het aantal puistjes, de fractie zieke bladeren en de fractie ziek bladoppervlak

Het aantal puistjes meeldauw per blad en de fractie zieke bladeren werden bepaald in praktijkpercelen en veldproeven met wintertarwe. De fractie ziek bladoppervlak kon worden afgeleid uit het aantal puistjes. Het verband tussen de fractie zieke bladeren en de fractie ziek bladoppervlak werd bestudeerd. Bij aanname van een constante blad- en puistjesgrootte kan een blad maximaal M_m puistjes herbergen. Als het aantal meeldauwkolonies (elk bestaande uit n puistjes) een binomiale kansverdeling volgt, dan kan het verband tussen de fractie zieke bladeren (I) en de fractie ziek bladoppervlak (S) worden beschreven door: $(1 - S) = (1 - I)^{n/M_m}$. De overeenkomsten van dit model met andere modellen wordt besproken en schattingen van parameters worden vergeleken. Het model verklaart hoe blad-, puistjes- en koloniegrootte het verband tussen de fractie zieke bladeren en de fractie ziek bladoppervlak beïnvloedt. Het model beschreef het verband tussen de metingen goed maar niet volledig.

De negatieve binomiaal beschreef het verband tussen de metingen slecht. Het model van Nachman gaf een goede beschrijving van het verband tussen het gemiddeld aantal puistjes kper blad ($\overline{m}$) en de fractie zieke bladeren (I). Voor de praktijkpercelen werd dit verband beschreven door: $\ln(\overline{m}) = 1{,}48 + 1{,}14 \ln(\ln[1/(1 - I)])$. De invloed van jaren, rassen, gewasstadia en bladpositie op het verband was niet significant.

De fractie zieke bladeren kan gebruikt worden om het gemiddeld aantal puistjes per blad te voorspellen, maar deze methode is minder efficiënt dan een directe telling van het aantal puistjes. Schattingsfouten van het voorspelde aantal puistjes per blad worden gegeven.

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