

## Incidence and Severity Relationships of Secondary Infections of Powdery Mildew on Apple

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### ABSTRACT

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Severity (amount of leaf tissue affected by disease) can be determined from incidence (proportion of leaves diseased) for secondary infections of apple powdery mildew (caused by *Podosphaera leucotricha*) on apple (*Malus pumila*) leaves. A simple relationship was derived in which  $\sqrt{\text{severity}}$  was proportional to incidence. The equation was developed iteratively although a similar solution was arrived at by making the variance of incidence and severity data independent of their respective means. The

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relationship remained constant for data from different locations or cultivars, but it varied according to different seasons. The relationship remained relatively constant for leaves of different age although younger leaves developed the most disease. Mildew severity can be derived by measuring incidence on the five to 10 youngest terminal leaves. For quantitative severity assessment, adjustment must be made for differing apple cultivars and/or growing seasons.

The relationship between proportion of plant units diseased (incidence) and the amount of plant tissue affected by disease (severity) is a valuable tool for disease assessment and management. In most cases it is considerably easier to determine the incidence of a disease rather than its severity; nevertheless, disease severity often is the preferred measurement because it describes the relative area of plant tissue infected. Although the area of infected tissue is the most commonly used measurement of severity, other measurements, such as number of lesions, can be used, especially when lesions are relatively uniform in size. Attempts have been made to relate incidence to severity for coffee rust (13) as well as powdery mildew and leaf rust of wheat (11). In both instances severity was considered to increase logarithmically with arithmetically increasing incidence. James and Shih (11) were able to show that the relationships for wheat powdery mildew and leaf rust were constant over a large geographical area, but varied with differing seasons or leaf positions. Little is known about the incidence/severity (I/S) relationship in other diseases.

Field assessment of secondary infection of apple (*Malus pumila* Miller) by powdery mildew (*Podosphaera leucotricha* [Elli. and Ev.] Salm.) is difficult under New York growing conditions. In contrast with distinct lesion formation under prolonged humid conditions in the orchard or greenhouse, secondary powdery mildew in the field typically occurs as an expansive and rather diffuse lesion that is often more apparent on the abaxial leaf surface. A recommended method of assessment for fungicide efficacy involves determining both incidence and severity (10) although other methods are used (2,6,8). Barlow (3) and Butt and Souter (5) have suggested the use of incidence to predict severity, and a simplified procedure for field assessment on which to base management decisions has been developed (4).

The initial intent of this study was to determine a simplified assessment procedure by which apple powdery mildew severity could be predicted from incidence data. Further elucidation of the I/S relationship became possible as the data were analyzed. Preliminary results of this study have been reported previously (14).

### MATERIALS AND METHODS

Data were collected from mildewicide evaluation orchards for the years 1969 through 1975. There were three locations: an orchard located at Geneva, NY containing 10-yr-old McIntosh and

Idared cultivars on MM.106 rootstock and Cortland on M.7 rootstock; an orchard located at Geneva containing 50-yr-old McIntosh and Cortland cultivars on seedling rootstock, and an orchard located at Sodus, NY 10 km south of Lake Ontario containing 50-yr-old Cortland on seedling rootstock. Mildew evaluations were made in August of each year by collecting 15–25 vegetative terminals from each tree. Individual leaves represented the sampling unit and they were rated for the absence or presence of mildew and the number of secondary lesions when mildew was present. A maximum lesion count of 10 was given to heavily infected leaves. The data were reduced prior to analysis by summing the lesion counts for each leaf position on all terminals of a tree and dividing by the total number of leaves observed on the tree. Thus, the final data represented average number of lesions (severity) and the proportion of infected leaves (incidence) for each tree. A total of 319 trees were analyzed according to year, location, and cultivar.

The data also were analyzed according to leaf position on the terminal. Because of varying numbers of leaves per terminal and to simplify data analysis, the leaf positions were classified into five regions. Region I consisted of the five oldest leaves (positions 1–5); region II to IV were progressively younger blocks of five leaves each, while region V consisted of the youngest leaves on terminals that had 21–28 positions (leaves).

Two methods of data analysis were utilized. Data were empirically transformed to achieve the best linearity between incidence and severity as measured by ordinary least squares or weighted least squares regression analyses. Tests for equal intercepts and parallel slopes were used to compare variation within years, locations, cultivars, and leaf positions (12), and when significantly different ( $P = 0.01$ ) the intercepts or slopes were separated according to a multiple comparison test for regression coefficients ( $P = 0.01$ ) (7). The second procedure transformed both incidence and severity values to achieve independence between the data means and variance prior to regression analysis. This was done because natural populations (ie, lesions or infected leaves) often are not randomly distributed, and a method of transforming data to account for non-random cases was used (15).

### RESULTS

Severity, as measured in this study, was the average number of lesions per leaf per tree rather than the actual area of diseased tissue. Incidence was determined by the proportion of infected leaves per tree. Considering all years, locations, cultivars, and leaf

positions, the I/S relationship was curvilinear and had a greater variance as incidence increased (Fig. 1A).

**Empirical transformation.** The simplest type transformation to linearize the relationship was a power function:

$$S^k = b + cI \quad (1)$$

in which  $S$  is severity,  $I$  is incidence,  $k$  is a real number,  $b$  is the intercept coefficient, and  $c$  is the slope coefficient. A best fit  $k$  of 0.49 was calculated by simple iteration based on test statistics of maximum coefficient  $t$ -ratios, minimum standard deviation about the regression, and randomly distributed residuals. However, we chose to use  $k = 0.50$  (ie,  $\sqrt{S}$ ) to simplify the relationship (Fig. 1B). We allowed an intercept term ( $b$ ) in Eq. 1 because we could not justify its exclusion on inductive grounds as did James and Shih (11). The I/S relationship still had an increasing variance with increasing incidence. Because further transformation of the data did not make the variance more uniform, a weighting procedure was used to achieve the best regression equation (12). Residual analyses indicated an appropriate weight was  $1/\hat{S}$ ; ie, the inverse of the predicted severity value when using ordinary least squares. All further computation utilized the weighted least squares method.

The general regression equation based on all data was

$$S^{0.50} = 0.25 + 2.34I$$

It has a standard deviation of  $S$  about the regression line of 0.224, the coefficient of determination ( $R^2$ ) was 92.3% and both

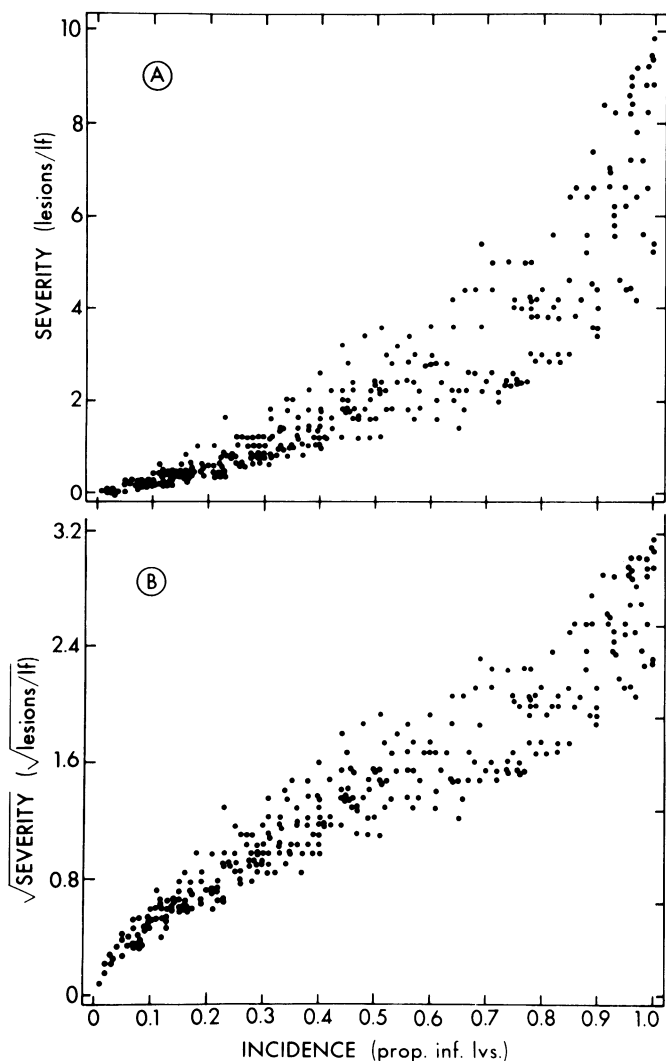


Fig. 1. A, Powdery mildew of apple. Relationship between severity (expressed as lesions per leaf) and incidence (expressed as proportion of leaves infected). B, Relationship between  $\sqrt{\text{severity}}$  and incidence.

coefficients were highly significant. Similar equations were derived for data within each parameter set; year, location, cultivar, and leaf position (Table 1).

The I/S relationship varied according to the particular year (Table 1). The intercepts for 1969 and 1973 were significantly lower than those for 1970–1972 and 1975 while 1971 had a significantly lower slope compared to all other years except 1970. The I/S relationship was not affected by orchard location or cultivar. Leaf position on the terminal had a significant, though random, effect on both the slope and intercept coefficients. Due to the varying sample size and coefficient variance, the intercept and slope of leaf positions 1–5 was significantly different from positions 6–10 and 11–15, although it was not different from positions 16–20 and 21–28. Fig. 2 shows the effect of cultivar and leaf position on incidence (Fig. 2A) and severity (Fig. 2B).

**Variance independence.** Neither the incidence nor severity data (Fig. 3) were normally distributed. The incidence data tended to be uniformly distributed, but the severity data tended to be skewed to the left. Taylor (15) has suggested transforming data to a new variable so the mean ( $m$ ) is independent of the variance ( $\text{var}$ ) by:

$$f(m) = m^{1-(b/2)} \quad (2)$$

where  $b$  is a population statistic indicating the randomness of the data. The  $b$  is derived from the mean and variance according to:

$$(\text{var}) = am^b \quad (3)$$

or

$$\ln(\text{var}) = \ln a + b \ln m \quad (4)$$

in which  $a$  is a population constant. In this study  $m$  was the incidence or severity value for each tree and  $\text{var}$  was the variance among leaf positions on the tree. According to Eq. 4 for incidence,

TABLE 1. Comparison of coefficients from regression lines of the form  $\sqrt{\text{severity}} = b_0 + b_1 \text{ incidence}$  for apple powdery mildew grouped by year, location, cultivar, and leaf position. The last two lines compare the weighted regression equation with the mathematically derived equation,  $\text{severity}^{0.45} = b_0 + b_1 \text{ incidence}$

Parameter	Number of trees	Intercept ( $b_0$ )	Slope ( $b_1$ )	$R^2$ (%) <sup>x</sup>
1969	47	0.11 a <sup>y</sup>	2.53 a <sup>y</sup>	96.3
1970	9	0.36 b	2.33 ab	97.5
1971	49	0.31 b	1.86 b	95.8
1972	21	0.27 b	2.43 a	96.6
1973	35	0.03 a	2.61 a	93.0
1974	94	0.26 b	2.45 a	96.0
1975	63	0.22 ab	2.87 a	94.9
Orchard 1	250	0.23 a	2.35 a	92.6
Orchard 2	44	0.25 a	2.35 a	91.9
Orchard 3	25	0.31 a	2.54 a	95.2
McIntosh	93	0.23 a	2.17 a	93.2
Idared	20	0.25 a	2.31 a	95.5
Cortland	206	0.27 a	2.39 a	91.8
Leaf 1–5 <sup>z</sup>	192	0.12 a	2.64 a	90.5
Leaf 6–10	319	0.16 b	2.36 b	93.7
Leaf 11–15	319	0.18 b	2.32 b	93.2
Leaf 16–20	317	0.11 ab	2.46 ab	92.7
Leaf 21–28	106	0.05 ab	2.38 ab	90.6
General equations				
Empirical trans.	319	0.25 a	2.34 a	92.3
Variance ind.	319	0.32 b	2.07 b	92.0

<sup>x</sup>Coefficients of determination ( $R^2$ ) are only approximate due to the weighted least squares procedure.

<sup>y</sup>Coefficients within each parameter group, followed by the same letter are not significantly different from each other based on a coefficient comparison test ( $P = 0.01$ ) (7).

<sup>z</sup>Terminals divided into regions based on leaf position. 1 = oldest leaf.

$b = 0.04$  and for severity  $b = 1.10$ . Using Eq. 2, the data were transformed by  $I^{0.979}$  and  $S^{0.449}$ . However, the incidence  $b$  was not significantly different from 1.0 so incidence was not transformed. Thus,

$$S^{0.449} = cI^{1.0} \quad (5)$$

The regression equation coefficients are presented in the last entry of Table 1.

### DISCUSSION

The I/S relationship of foliar plant diseases is a recognized, but little utilized, epidemiological phenomenon (3,11,13). Disease assessment in large field experiments or surveys can be made more manageable if a clear relationship between incidence and severity can be established, thus requiring only incidence data to establish severity levels. While this method may adversely affect assessment precision (the deviation from the mean obtained from repeated samples) it can actually improve the assessment accuracy (deviation from the true mean of the population) since more samples can be assessed and there is a better likelihood of accounting for nonrandom disease distribution.

A general I/S relationship for a disease such as apple powdery mildew cannot be automatically applied to all occurrences of the disease. We undertook this study to determine a general I/S relationship for apple powdery mildew and then to establish whether that relationship would remain the same for different years, locations, cultivars, or leaf positions. Logarithmic I/S relationships had been established for coffee rust (13) and powdery mildew leaf rust of wheat (11). The I/S on wheat was patterned after Gregory's multiple infection equation (9). The simple I/S relationship described in Eq. 1 seems to be more logical and flexible. The exponent  $k$ , while linearizing the I/S relationship, becomes an index comparable to other diseases or epidemiological conditions. A  $k$ -value of 0.5 was empirically chosen for apple powdery mildew. Raising  $S$  to the 0.5 power ( $\sqrt{S}$ ) still did not provide a homogeneous variance (Fig. 1B), so a weighted least squares method was used. Data values closer to 0, where the I/S relationship was more clearly defined, were given greater weight than data values closer to maximum  $I$  or  $S$  where much more variation in the observed data occurred. The resulting regression equation accounted for 92.3% of the data variation.

Data separated according to the location parameter showed no significant difference between the regression coefficients for each orchard (Table 1). Although the orchards were up to 40 km apart, it appeared that within a climatologically similar region, I/S relationships should be approximately the same. Similarity of I/S relationships between locations also has been reported for wheat diseases (11). The location data also suggest there may be no significant effect of tree age and rootstock on the I/S relationship. More data are needed to precisely determine this effect.

Different cultivars had no effect on the regression coefficients of the I/S regression line (Table 1). Although Cortland is more susceptible than McIntosh and Idared has an intermediate susceptibility, the relationship between incidence and severity remains constant for each cultivar.

Regression coefficients for data separated according to leaf position on the terminal also varied (Table 1). The only significant coefficient difference was between leaf positions 1-5 and 6-15 ( $P = 0.01$ ). The slope coefficients were remarkably similar considering the fact that average incidence and severity values generally were increasing with increasing leaf position (Fig. 2). This indicated the true linearity of the  $k = 0.5$  transformation since the slope for leaf position group 1-5, with a narrow data range, was the same as the slope for leaf position group 16-20, with a wide data range.

Separation of data according to year provided the largest differences between regression coefficients and produced the highest  $R^2$  values compared to other types of data separation. This indicated that seasonal variability had the greatest effect on altering the I/S relationship. When the effect was removed by calculating an I/S relationship for each year, variability was reduced and the test

statistics greatly improved (Table 1). James and Shih (11) also found seasonal differences within their data.

Incidence and severity values increased as leaf age decreased (Fig. 2). Because a single mildew assessment was made after terminal growth ceased, the  $I$  and  $S$  values for each leaf position are

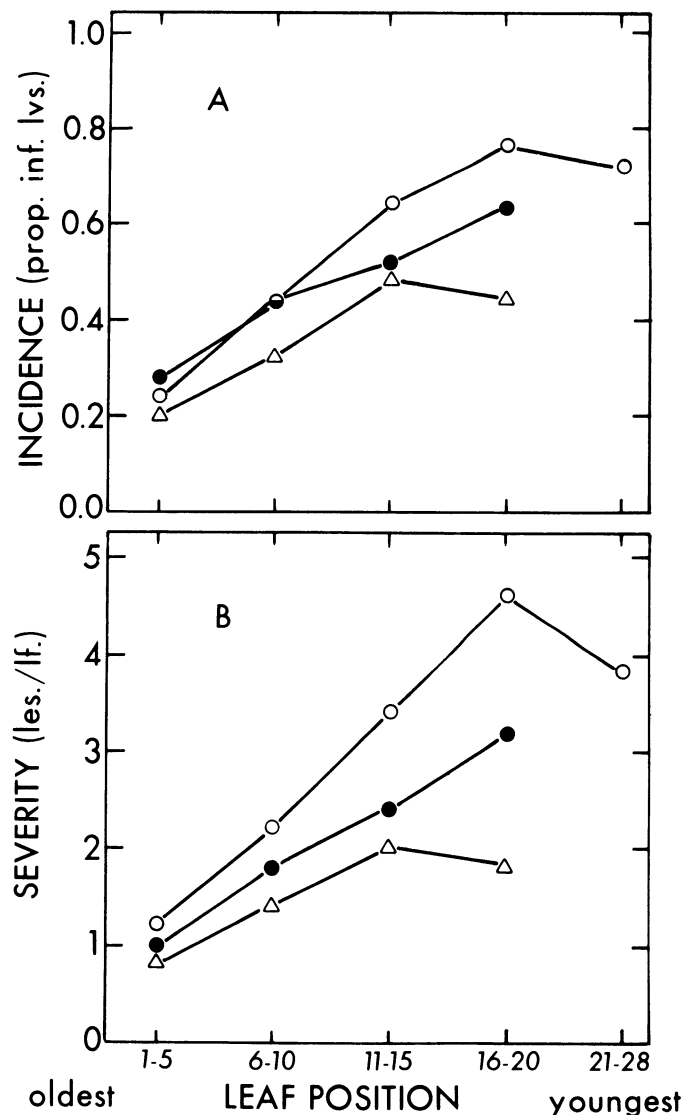


Fig. 2. Powdery mildew severity and incidence of three apple cultivars on leaves at different positions on the terminal (1 = oldest leaf). A, Incidence (expressed as proportions of leaves infected). B, Severity (expressed as lesions per leaf). Legend: ○ = Cortland, ● = Idared, and △ = McIntosh. Only Cortland terminals extended beyond 20 leaf positions.

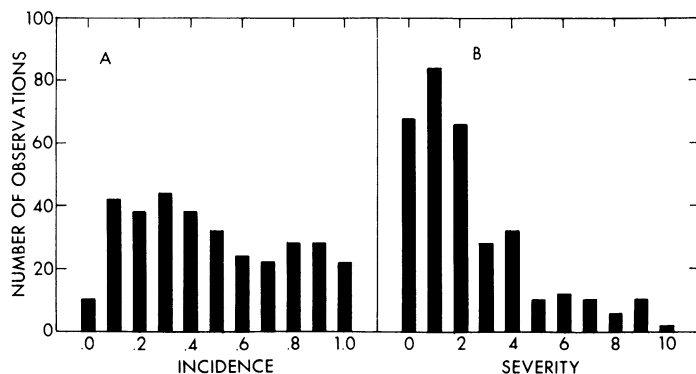


Fig. 3. Powdery mildew of apple. Observation frequency of A, Incidence (proportion of leaves infected) and B, Severity (average lesions per leaf).

comparable. Averaged over the seven years, the I and S values are highest on the terminal ends. Separation of cultivar also is maximized at the terminal ends. While the exact terminal length varies, and thus, the position number of the youngest leaf, the data show that an assessment procedure involving the five or 10 youngest leaves on a terminal would provide a reliable mildew measure.

Although our preliminary data indicate that fungicides affect the I/S relationship (14), this aspect must be examined in more detail.

The results achieved by the variance independence solution is very similar to the empirical solution. This is particularly true because the incidence data means were initially independent of their variance and required no transformation. The coefficients of the two equations are not similar (Table 1) because of the different S transformations. The comparisons of coefficients of data separated according to year, location, cultivar, or leaf position would have been the same if  $S^{0.45}$  was used instead of  $S^{0.5}$ .

An additional conclusion can be made about the b values obtained from Eq. 3 for incidence ( $b = 0.04$ ) and severity ( $b = 1.10$ ). At  $b = 1$  the population is random, while as  $b \rightarrow 0$  the distribution becomes more uniform and as  $b \rightarrow \infty$  (usually no greater than 3.0) the distribution becomes highly aggregated (15). This is somewhat analogous to Blackman's ratio as presented by Gregory (9). The incidence of all levels of powdery mildew tends to be quite uniform so that one can expect to find the same number (approximately) of trees with 20% infected leaves as trees with 50 or 80% infected leaves (Fig. 3A). On the other hand mildew lesions tend to be slightly aggregated. Lesions of other apple pathogens, *Venturia inaequalis* (1) and *Gymnosporangium juniperi-virginianae* (9), also are aggregated.

As a further verification of Eq. 1 with  $k = 0.5$ , two published sets of data were fitted to the equation. Hickey (Table 1 in Ref. 10) listed a small data set ( $n = 7$ ) with both incidence and severity for apple powdery mildew on the cultivars Jonathan and Rome Beauty. Regression of S vs. I yielded  $R^2$  of 80.3 and 93.7% for Jonathan and Rome Beauty, while regression of  $S^{0.5}$  vs. I yielded  $R^2$  of 93.9 and 99.6%, respectively. Data on wheat powdery mildew from James and Shih (Fig. 5 in Ref. 11) were extracted from the plot of severity vs. incidence with an electronic digitizer and then regressed. The James and Shih equation for S when  $I \leq 65\%$  and Eq. 1 each accounted for the same amount of data variation. However, when the whole data set ( $I \leq 95\%$ ) was used, the James and Shih equation accounted for 70% of the data variation while Eq. 1 accounted for 81% of the data variation. The method of severity measurement does not seem to affect I/S relationship because severity was measured as lesions per leaf in this study and a percent leaf area

infected in the two examples (10,11). Therefore, although the coefficients are not directly comparable, because of measurement technique, there appears to be a generalized relationship between incidence and severity for at least two powdery mildew genera.

If quantitative severity values are desired from incidence data several steps should be followed: first, determine appropriate methods of incidence and severity measurement, second, obtain preliminary incidence and severity data to establish the proper coefficient values for each season, and third, determine incidence for the remaining observations and convert to severity.

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