Apple Powdery Mildew Disease Progress on Sections of Shoot Growth: An Analysis of Leaf Maturation and Fungicide Effects

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Contribution 1437, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication as Journal Series Paper 6855.

This material is based upon work supported by the Environmental Protection Agency Grant CR-806277-020 and by the U.S. Department of Agriculture under agreement 71-59-2481-1-2-039-1. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

Accepted for publication 23 July 1984.

ABSTRACT

Lalancette, N., Jr., and Hickey, K. D. 1985. Apple powdery mildew disease progress on sections of shoot growth: An analysis of leaf maturation and fungicide effects. Phytopathology 75:130-134.

The separate and combined effects of leaf aging and fungicide application on the incidence of apple powdery mildew were studied by monitoring disease progress on sections of shoots. In 1980, 1981, and 1982 six cultivar Rome Beauty trees were sprayed to run off at 2-wk intervals with the fungicide bitertanol at 150 mg ai/L; six unsprayed trees were used as controls. Vegetative terminals equally spaced around the periphery of each tree were chosen for observation. The youngest, most-unfolded leaf was tagged during each of the first three of five disease assessments made in each year; thus, by the end of the season three sections of shoot were demarcated. Analysis of disease incidence on each section revealed that disease severity first increased then decreased. Although bitertanol significantly reduced the rate of disease increase during the early phase of the epidemic, further reduction in disease incidence during the later phase was attributed to leaf maturation. We postulate that an increase in resistance during leaf aging deleteriously affects established fungal colonies. Approximately 46 and 39% of the untreated leaves harboring colonies during the early stages of the epidemics in 1980 and 1981, respectively, were no longer visibly infected by the last assessment.

Additional key words: *Podosphaera leucotricha*.

Secondary inoculum production by the powdery mildew fungi is critical for disease development (4). Sterol-inhibiting fungicides and host resistance, including changes that occur during tissue aging or maturation, may influence sporulation (7,10). However, unlike fungicidal control, less information is available about how age-related resistance affects disease progression.

The immature foliage of apple is known to be the tissue that is most susceptible to *Podosphaera leucotricha* (Ellis and Everhart) Salmon (3). The inoculation of leaves of different ages results in fewer lesions on older leaves (1); colonization and sporulation can also be impaired as tissue ages. Recognition of mildew colonies on older leaves is often difficult, indicating that sporulation declines with leaf age (5). Although the sterol inhibitor bitertanol suppresses fungal growth (2), the reduction in sporulation attributed to successive fungicide applications may also be a function of increasing resistance with leaf aging. It is also possible that the fungicide and resistance of older tissue complement each other. Recognition of the relative importance of these two factors would improve our understanding of the epidemiology of apple powdery mildew.

The objective of this study was to examine the separate and combined effects of leaf age and fungicidal control by observing disease progression on sections of shoots, each section consisting of a set of leaves of similar age.

MATERIALS AND METHODS

Field design. In 1980, the field layout consisted of four rows of "tree groups," each row 20 tree groups long. A "tree group" included one tree each of cultivars Rome Beauty, Stayman Winesap, and Delicious grafted onto M7 semidwarfing rootstock and planted at the same location in 1971. Each tree group, spaced at 9 m × 10.7 m, was pruned as if it was a single tree, each cultivar occupying about one-third of a group. In 1981 and 1982, the orchard design consisted of four rows each with 12 standard trees of Rome Beauty on seedling rootstock. Spacing was 9 × 10.7 m and the trees were pruned to moderate density.

Fungicide treatment. The 80 tree groups in the 1980 experiment were divided into six blocks of approximately rectangular shape; 14 tree groups were missing. Bitertanol (Baycor 50W, Mobay Chemical Corp., Kansas City, MO) was applied at 150 mg ai/L to the point of run off to one tree group randomly selected within each block. Starting on 22 April, applications were made at 7-day intervals before petal fall and at 14-day intervals after petal fall. One unsprayed control tree group was also randomly selected within each of the six blocks.

During bloom in 1981 and 1982, the total number of shoots and clusters with overwintering (primary) mildew was counted for each tree. Six trees were then selected ranging from high to low levels of primary mildew. Bitertanol (Baycor 4F) was applied at 150 mg ai/L to the point of run off to the six trees. Applications were made at 14-day intervals, starting on 6 May in 1981 and on 10 May in 1982. Six unsprayed trees similarly chosen served as controls.

Disease assessment. To assess disease in 1980, five vegetative terminal shoots were selected and tagged on the Rome Beauty cultivar within each tree group. In the 1981 and 1982 experiments, 10 vegetative terminals were selected and tagged on each tree. Selected shoots were equally spaced around the periphery of each tree. In all years, disease was assessed five times at 14-day intervals. During each of the first three observations, the youngest, most-unfolded leaf on each terminal was tagged. Thus, by the end of the season three sections of shoot were demarcated (Fig. 1). During each assessment, the number of visibly diseased leaves, observed without magnification, and the total number of leaves on the most recent and previous sections were recorded. From these data, the proportion of infected leaves for each section was calculated.

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Data analysis. An analysis of variance was performed for each growth interval in each year to determine the effect of fungicide treatment and time or leaf aging on disease incidence. In 1980, the six blocks were included in the model as a statistical blocking factor, but in 1981 and 1982 this factor was replaced by the level of primary mildew. In both cases, this blocking factor was considered a random effect, while the fungicide and time factors were fixed effects. Contrasts of disease incidence means among different shoot sections, the control and fungicide treatments, and the various times of disease assessment were performed by using multiple t-tests. Significance levels for each set of "s" contrasts in each analysis of variance were calculated by α/s, in which $1-\alpha = 0.90$ was the Bonferroni overall confidence coefficient (9).

RESULTS

Disease-increase phase. The relatively high amounts of disease incidence observed for the first assessment on shoot sections two and three implied that a considerable proportion of infection occurred during the 2 wk prior to those counts (Fig. 2). This was the period during which leaves were being produced and, hence, were immature. The difference between the control and treated section was significant in each year for sections two and three, except for section two in 1980 (Table 1).

Disease did not occur on section one for the first assessment in 1981 and 1982 because this assessment was made just prior to onset of the epidemic (Fig. 2C to F). Hence, the difference between the control and fungicide treatment for section one was nonsignificant (Table 1). In 1980, however, the first assessment on this section was performed much later in the epidemic and after several fungicide applications. Thus, disease was observed during the first assessment on section one in 1980 (Fig. 2A and B) and the control and untreated sections were significantly different (Table 1).

During the 2-wk period between the first and second assessments on most shoot sections, an additional increase in disease incidence was also evident for both treatments (Fig. 2). This increase could be attributed to latent infections not observed until the second assessment, in addition to new infections occurring between the first and second assessments. During this period, however, four of the seven untreated sections had a rate of disease increase not significantly different from those of the corresponding treated sections (Table 2). Thus, the effect of the fungicide was less pronounced as leaves became less susceptible with age.

Disease-decrease phase. In 1980 and 1981, the rate of disease decrease was similar for both the bitertanol-treated and untreated sections; only one of the contrasts was significant (Table 3). The reduction in the proportion of infected leaves resulted from a reduction in the number of infected leaves because the total number of leaves constituting each section was constant over time. Approximately 46 and 39% of the leaves of untreated sections found infected on the second assessment in 1980 and 1981, respectively, were no longer visibly infected by the last assessment (Table 4). Similar reductions of 41 and 51% were observed for the treated sections in 1980 and 1981, respectively. This apparent disappearance of lesions, as indicated by a reduction in the frequency of infected leaves, was significant for five of the 10 year × treatment × section combinations (Table 4). A severe scab infection in 1982 caused many untreated leaves to abscise and, hence, prevented a final assessment on every shoot section.

Interactions. Both the fungicide factor and time (leaf age) factor were significant sources of variation for disease incidence (Table 5). The spatial blocking factor was significant for only the third section in 1980, while the effect of incidence of primary mildew as a

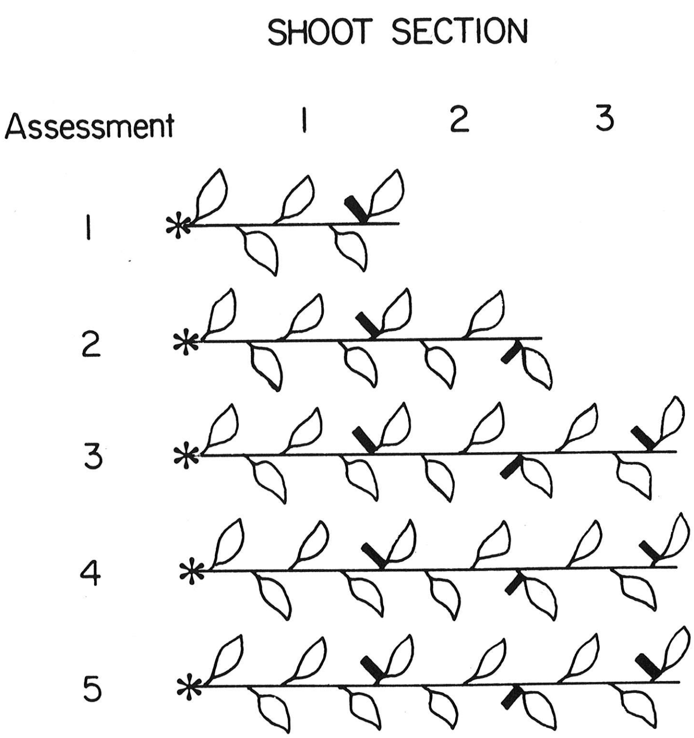

Fig. 1. Tagging procedure on a typical vegetative shoot allowing separation of increments of growth. Each shoot section consisted of leaves of similar age produced during a 2-wk growth period. The asterisk indicates position of the terminal bud scar.

TABLE 1. Comparison of the control and bitertanol treatments for the first assessment of apple powdery mildew on the leaves of three successive apple shoot sections

Year	Shoot section[a]	Proportion of infected leaves: Control	Bitertanol	Difference	$P(T>\|t\|)$[b]
1980	1	0.2447	0.0591	0.1856	0.0001*
	2	0.2228	0.0411	0.1817	0.0366
	3	0.5185	0.3295	0.1890	0.0037*
1981	1	0.0056	0.0042	0.0014	0.8882
	2	0.2822	0.1569	0.1253	0.0017*
	3	0.5865	0.3504	0.2361	0.0001*
1982	1	0.0038	0.0032	0.0006	0.9567
	2	0.3938	0.2339	0.1599	0.0009*
	3	0.7243	0.1799	0.5444	0.0001*

[a] Section 1 is the oldest; see Fig. 1.
[b] To provide a yearly overall confidence coefficient of 0.90, probabilities less than 0.0077, 0.0083, and 0.0167 in 1980, 1981, and 1982 comparisons, respectively, indicate a significant difference and are denoted by an asterisk (*).

TABLE 2. Comparison of the control and bitertanol treatments for the rate of increase of powdery mildew on the leaves of apple shoot sections between assessments one and two

Year	Shoot section[a]	Proportion of leaves infected/day: Control	Bitertanol	Difference	$P(T>\|t\|)$[b]
1980	1[c]	...	...	...	...
	2	0.0348	0.0158	0.0190	0.0306
	3[c]	...	...	...	...
1981	1	0.0053	0.0015	0.0038	0.0010*
	2	0.0176	0.0084	0.0092	0.0065*
	3	0.0100	0.0076	0.0024	0.3822
1982	1	0.0062	0.0039	0.0023	0.0715
	2	0.0243	0.0063	0.0180	0.0003*
	3	0.0146	0.0100	0.0046	0.2871

[a] Section 1 is the oldest; see Fig. 1.
[b] To provide a yearly overall confidence coefficient of 0.90, probabilities less than 0.0077, 0.0083, and 0.0167 in 1980, 1981, and 1982 comparisons, respectively, indicate a significant difference and are denoted by an asterisk (*).
[c] Except for the bitertanol-treated shoot section 3, no disease increase was observed on these sections between assessments one and two (see Fig. 2A and B).

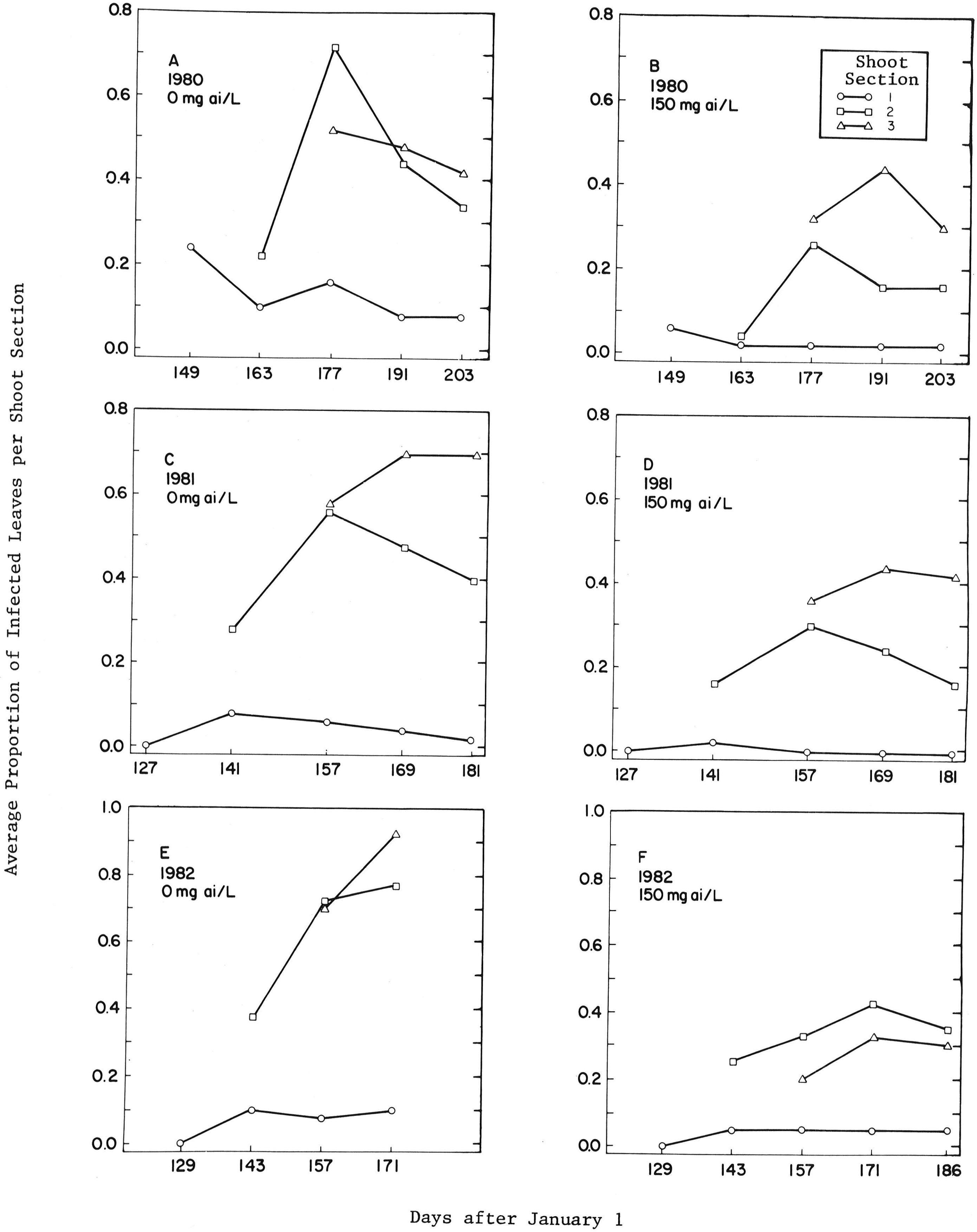

Fig. 2. Progression of apple powdery mildew disease on untreated and bitertanol-treated sections of shoot growth in 1980, 1981, and 1982. Each shoot section consisted of a set of Rome Beauty leaves of similar age produced during a 2-wk period, such that section 1 had the oldest leaves while sections 2 and 3 were progressively younger. Disease incidence was calculated by averaging over all shoot sections per tree and trees per treatment.

blocking factor was highly significant for all but one section in 1981 and 1982.

Five of the nine shoot section × year combinations had significant fungicide × time interactions (Table 5). That is, the difference between the treated and untreated sections varied significantly according to the time of assessment. Comparison of the disease progress curves for growth segments one and two in 1981 revealed that this interaction occurred during the disease increase phase (Fig. 3).

The significant fungicide × block interactions in 1981 and 1982 indicated that the difference between treatments varied over different levels of initial inoculum. The level of secondary mildew was dependent on the combined interactive effect of the fungicide and primary mildew factors. The nonsignificant time × block interactions suggested that differences in the amount of secondary mildew attributed to different spatial blocks or levels of primary mildew did not vary significantly over the course of the epidemic.

DISCUSSION

The progression of powdery mildew on foliage of similar age was demonstrated to be a function of fungicide application and leaf age. Although bitertanol has been reported to have protective, curative, and eradicative properties (2), its primary effect on disease incidence was as a protectant fungicide. Bitertanol significantly reduced disease incidence during the period when leaves were young and most susceptible. That is, the fungicide prevented new infections by reducing infection efficiency and/or sporulation. However, the fungicide was not responsible for the eradication of lesions later in the epidemic, indicating the presence of another contributing factor, leaf aging or maturation.

Most experiments designed to study the effect of changing host susceptibility with time focused on the ability of the pathogen to cause an infection (10). For example, studies have shown that *Venturia inaequalis* initiates the greatest number of lesions on the youngest, most immature apple leaves (11). Although the present study showed that *P. leucotricha* similarly infected younger leaves to a greater degree, the results also indicated that an increase in resistance with age had a deleterious effect on lesions already present. Fungal colonies observed during the early phase of the epidemic were no longer macroscopically visible toward the later stages.

The "disappearance" of mildew lesions was attributed to characteristics peculiar to mildew diseases and to the conditions of the epidemic. In all 3 yr the severity of mildew was low; most leaves were infected by only a single lesion. Furthermore, little if any of the typical symptoms such as leaf curling, crinkling, and elongation were evident. Thus, disease incidence assessment was based solely on the presence or absence of signs: the superficial mycelium, conidiophores, and conidia. Given that infection decreases with leaf age (1,3), then possibly colonization and sporulation are also reduced if not entirely halted as a leaf matures. We hypothesize that the mycelium, conidiophores, and conidia eventually die and desiccate, leaving behind an apparently healthy leaf without any signs or symptoms. Consequently, subsequent assessments on the same set of leaves revealed a decrease in the proportion of infected leaves. Had new, susceptible growth been included in each successive assessment, the reduction in disease incidence on older leaves may well have been offset by new infection on young leaves. Further experimentation conducted at a greater disease severity and involving microscopic examination of lesions is needed to substantiate this hypothesis.

Although weather variables no doubt have an influence in promoting infection (8), the results indicated that the loss of mildew lesions may not be related to weather. In 1981, disease incidence was decreasing on shoot section one under the same weather conditions in which disease was increasing on section two. At a later time, disease was then decreasing on section two while increasing on section three. Simultaneous increase and decrease of

TABLE 3. Comparison of the control and bitertanol treatments for the rate of decrease of powdery mildew on the leaves of apple shoot sections between the second and last assessments

Year	Shoot section[a]	Proportion of infected leaves/day: Control	Bitertanol	Difference[b]	$P(T>\|t\|)$[c]
1980	1[d]	−0.0032	−0.0008	0.0024	0.0045*
	2	−0.0139	−0.0040	0.0099	0.0369
	3	−0.0043	−0.0106	0.0063	0.3133
1981	1	−0.0014	−0.0005	0.0009	0.0168
	2	−0.0071	−0.0053	0.0018	0.3690

[a] Section 1 is the oldest; see Fig. 1.
[b] Absolute value of the difference between the control and bitertanol rates.
[c] To provide a yearly overall confidence coefficient of 0.90, probabilities less than 0.0077 and 0.0083 in 1980 and 1981 comparisons, respectively, indicate a significant difference and are denoted by an asterisk (*).
[d] Rate of decrease between the first and last counts for section 1, 1980.

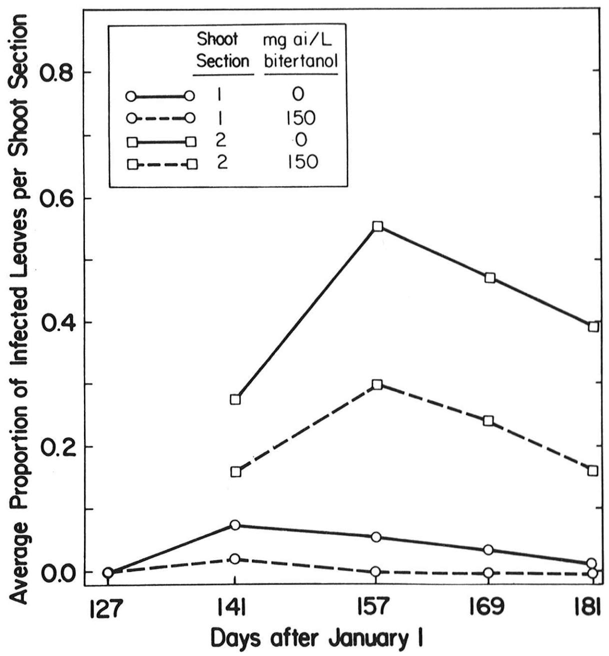

Fig. 3. Disease progression for untreated and bitertanol-treated shoot sections in 1981. Nonparallel lines during the disease increase phase indicated interaction of treatments over time. Parallel lines during the decrease phase indicated no interaction.

TABLE 4. Decrease in the frequency of infected leaves during the decrease phase in the epidemic of apple powdery mildew on successive shoot sections for treatments in 1980 and 1981

Year	Treatment	Shoot section[b]	Frequency of infected leaves[a]: Second assessment	Last assessment	Difference	$P(T>\|t\|)$[c]
1980	Control	1	71[d]	27	44	0.0001*
		2	86	44	42	0.0004*
		3	39	35	4	0.3264
	Bitertanol	1	20[d]	6	14	0.1406
		2	35	21	14	0.2022
		3	37	27	10	0.0297
1981	Control	1	42	13	29	0.0001*
		2	118	85	33	0.0001*
	Bitertanol	1	14	3	11	0.0623
		2	67	37	30	0.0014*

[a] Total number of infected leaves over all shoots on all trees.
[b] Section 1 is the oldest; see Fig. 1.
[c] *t*-tests performed on the average number of infected leaves per shoot per tree. To provide a yearly overall confidence coefficient of 0.90, probabilities less than 0.0077 and 0.0083 in 1980 and 1981 comparisons, respectively, indicate a significant difference and are denoted by an asterisk (*).
[d] First assessment.

TABLE 5. Significance probabilities[a] of F-values associated with an analysis of variance on the average proportion of apple leaves per shoot section infected with *Podosphaera leucotricha*

Source of variation	Year and shoot section[b] 1980 1	1980 2	1980 3	1981 1	1981 2	1981 3	1982 1	1982 2	1982 3
Fungicide	0.0019*	0.0003*	0.2943	0.0196*	0.0036*	0.0129*	0.0492*	0.0194*	0.0084*
Time	0.0013*	0.0001*	0.4400	0.0001*	0.0001*	0.0004*	0.0013*	0.0026*	0.0721
Block	0.2380	0.0515*	0.0001*	0.0001*	0.0001*	0.0001*	0.0796	0.0001*	0.0031*
Fungicide × Time	0.0340*	0.1024	0.1690	0.0041*	0.0340*	0.4947	0.0035*	0.0005*	0.2871
Fungicide × Block	0.1120	0.7149	0.0006*	0.0043*	0.0020*	0.0001*	0.1512	0.0489*	0.0717
Time × Block	0.2239	0.8236	0.0265	0.4010	0.8978	0.3233	0.1703	0.2064	0.0659

[a]Probabilities less than 0.05 indicate significant factors or interactions at the 0.95 confidence level and are denoted by an asterisk (*).
[b]Section 1 is the oldest; see Fig. 1.

disease on different sections was also observed for the treated shoots in 1980. Those sections with decreasing disease incidence were 2 wk older than those showing an increase; the effect of changing host susceptibility with age was considered the more probable cause. However, in 1980 and 1982 this pattern was not evident for most shoot sections.

The significant fungicide × time interaction has important implications for the evaluation of fungicides. An evaluation of treatments by examination of disease early in the epidemic will most likely lead to different results than a similar evaluation performed later in the epidemic. Butt and Barlow (5) recommended an assessment of disease on only the five youngest leaves per shoot, thus allowing successive assessments to be made on leaves of similar age, though at different times. Hickey (6) advocated an assessment of all the leaves on each shoot performed at the end of the epidemic. This method provides an overall assessment of the cumulative effect of the treatments over leaves of all ages, and therefore evaluates the combined effect of leaf age and fungicide treatment. Thus, the choice of technique will depend on whether the effects of leaf aging are to be held constant or included as part of the overall disease assessment.

In addition to the effects of leaf age and fungicide application, primary mildew was also shown to be an important factor in determining the incidence of secondary mildew. Although the role of primary mildew as a source of initial inoculum for the secondary mildew epidemic is well understood, more information is needed on the quantitative aspects of this relationship.

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