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### PROCEEDINGS-

# APPLE AND PEAR SCAB WORKSHOP

Kansas City, Missouri July 11, 1976

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The Apple and Pear Disease Workers
In Cooperation with
American Phytopathological Society
Cooperative State Research Service, U. S. Department of Agriculture
New York State Agricultural Experiment Station



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### MATURATION AND DISCHARGE OF ASCOSPORES OF THE APPLE SCAB FUNGUS

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Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is the most important disease of apple in humid-temperate climates. In such areas, the fungus overwinters primarily as mycelium in fallen infected leaves. Perithecia are produced during the winter in these leaves and, by spring, contain mature ascospores which are the primary source of inoculum for the scab disease each year. Control measures for scab rely on fungicide sprays during this period of ascospore production. Thus, an understanding of ascospore productivity, maturation, and release is fundamental to an effective and efficient control program.

#### ASCOSPORE MATURATION

An ascospore of *V. inaequalis* is considered mature when fully formed, has developed a light olive color, is capable of being discharged from its ascus when the leaf is wetted, and will germinate in water and thus has the potential for infection. All eight ascospores in an ascus appear to mature simultaneously, but the rate of maturity of different asci within a perithecium may vary considerably. Furthermore, maturation varies greatly among perithecia on the same or different leaves in the same orchard.

METHODS—Direct observations. Ascospore maturity can be determined by microscopic observation of the contents of crushed perithecia extracted from leaves. Numerous workers have used this method, but only Szkolnik (20) has published details of a routine procedure. He examined about 20 asci from each of at least 25 crushed perithecia taken from several leaves. Each ascus was categorized according to the maturation of the contents (Table 1). Palmiter (16) examined about 100 perithecia per sample and rated each for relative spore maturation (Table 1). These two methods provide comparable information of ascospore maturity and have been used routinely and successfully for over 25 years in grower advisory spray programs in two areas of New York State (16,20, Table 2). Because such methods are tedious, time consuming, and require the services of trained technicians, the number of samples which can be processed at one time is limited.

Mathematical prediction. Utilizing 17 years of data collected by Szkolnik in New York, Massie and Szkolnik (12,13) developed a predictive equation for ascospore maturity using multiple regression analysis. The equation shows that maturity depends to a large extent on the number of accumulated degree-days (base 32 F) from leaf

Table 1.—Categories used by Szkolník and Palmiter determining maturation of primary stage of apple scab fungus (*Venturla inae-gualis*) in overwintering leaves.

Category	Szkolnik - % Asci	Palmiter - % Perithecia
1	No spore formation evident	No spores evident
2	Spores forming	Colorless spores
3	Spores formed, not colored	10% colored spores <sup>a</sup>
4	Spores formed and colored	25% colored spores
5	Spores discharged	50% colored or discharge

a Colored spores are considered to be mature but not necessarily capable of being discharged.

Table 2.—Primary scab differential counts for Venturia inaequalls reported by Szkolnik and Palmiter in two different phenological areas of New York State in April 1962.

Category	Szkolnik (April 12) a	Palmiter (April 13)
1	74	16
2	21	55
3	7	17
4	2	10
5	0	2

a % in each category.

fall and to a much lesser extent on accumulated precipitation during the same period. This model, although not yet fully proved in practice, could be useful in predicting ascospore maturation in different macro- and micro-climates without the use of tedious visual determinations.

**OBSERVATIONS**—Usually ascospore maturity and the apple host development are closely correlated, whereas correlation of both events with calendar dates is low phenologically (24). In 16 years of observations, Szkolnik (21,22,23) found in New York State that some ascospores are mature by the apple flower green tip stage (4). Szkolnik's differential counts showed a general shift from immature to mature ascospores as the spring season progressed. The percentage of colored spores increased week by week until about bloom to the early petal fall stage

of 'McIntosh' cultivar development and then diminished. Late in the primary scab period, accurate counts were not possible because the remaining spores were usually abnormal, the empty asci had degenerated, and the perithecia and leaves were in various states of decomposition

In any year, Szkolnik (23) found that ascospore maturity varies from the average (Fig. 1). He observed mature ascospores as early as late February 1975 in New York following an unusually mild winter. This was 6 to 8 weeks earlier than average. In 1938, ascospores matured in midwestern United States about 3 weeks before normal, probably due to unusually warm temperatures in February (1). In some years, maturity may be delayed by a week or so compared to apple tree development (Fig. 1).

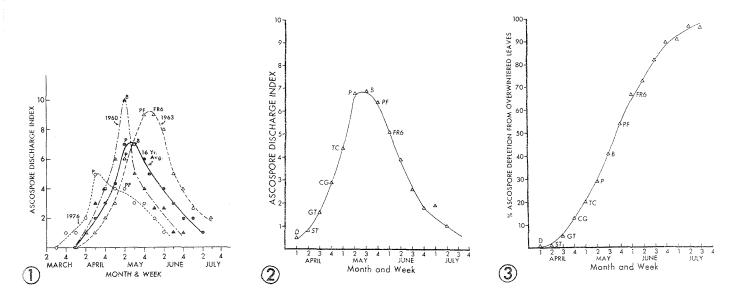
Other than temperature and moisture, the role of environmental factors on spore maturation is not understood. Light may be of significant importance in determining the daily periodicity of maturation (3). The time that leaves became infected the previous year, snow cover during winter months, apple cultivar, and similar factors are no doubt of importance.

#### ASCOSPORE DISCHARGE

Hirst and Stedman (8) defined 'ascospore productivity' as the number of spores produced per cm² of dead leaf area. They differentiated the latter from the term 'ascospore dose' which is the number of the spores per volume of air (per m³) caught in spore traps.

**METHODS**—Discharge of ascospores from moistened leaves has been observed and measured in water or air (Table 3). Brook (3) described the discharge of ascospores under the microscope from leaf pieces mounted on modeling clay. Before it discharged, each ascus emerged through the ostiole and extended until its tip was about 40 microns beyond the ostiole. All eight ascospores were discharged in rapid succession, and the spent ascus then shrank back into the perithecum. When completely submerged in water, asci required from 45-120 seconds to discharge, whereas in air the process took as little as 3.5 seconds.

Szkolnik (21) provided a method for the routine measurement of ascospore discharge in Petri plates in which moistened leaves were allowed to shoot spores into water with an intermediate air medium. Discharge was measured as the number of spores per hour. Hutton and Burchill (10) suspended leaf discs in 40 ml of water in a bottle and bubbled air through the water for 2 hours to keep the discs and spores in suspension. The filtered spore suspension then was concentrated by centrifugation and the spores counted in a hemocytometer. Ascospore productivity was recorded as spores/cm²of leaf surface area. Hirst and Stedman (8) and Brook (2) utilized a flat wind tunnel containing trays of infected leaf discs. Air drawn over the surface of the discs carried ascospores to slide impactors placed downwind. Spores were recorded as number/m³ of air. Gilpatrick et al. (6) modified the latter technique by using an upright tower. A simple method was used by Dunn (5) to monitor ascospore release. He merely placed greas-



Figures 1-3. Ascospore productivity of Venturia inaequalis. Fruit bud stages of apple: D=dormant; ST=silver tip; GT=green tip; CG=one cm green; TC=tight cluster; P+pink; B=bloom; PF=petal fall; FR6=fruit 6 mm diameter. (1) Sixteen-year average compared with three individual years of weekly monitoring of the abundance of apple scab ascospores released from freshly-collected overwintered McIntosh leaves in the Geneva, New York area. Ascospore index is the relative abundance of mature spores physiologically ready to discharge when the leaves become wet. (2) Sixteen-year average of weekly tests monitoring the abundance of apple scab ascospores released from freshly-collected overwintered McIntosh leaves in the Geneva, New York area. (3) Sixteen-year average of week-by-week depletion of apple scab ascospores from overwintered McIntosh leaves in the Geneva, New York area.

Table 3.—Some techniques for evaluating ascospore discharge (productivity).

Author and reference	Type Measurement		Other uses <sup>a</sup>		
Small (17)	Glass slide	Productivity	-		
Dunn (5)	Glass slide	Productivity	-		
Keitt and Jones (11)	Glass slide	Productivity	Seasonal release		
Brook (3)	Microscope	Release mechanism	-		
Szkolnik (21)	Petri plate	Productivity (Discharge index)	Phenology		
Hirst and Stedman (8);	Wind tunnel	Productivity	Environmental effects		
and Brook (3)	Wind tunnel		Phenology		
Gilpatrick et al. (6)	Wind tower	Productivity	Spore germination Treatment effects		
Hutton and Burchill (10)	Water bubble	Productivity	Treatment effects		

<sup>&</sup>lt;sup>a</sup>All methods are useful in spray forecasting.

ed slides on the surface of leaves on the orchard floor and made observations at appropriate times. Small (17) suspended a glass slide on a stake 18 inches above the ground and shielded the slide from rain with a metal cover.

OBSERVATIONS—Ascospore productivity of selected leaf samples has been studied by many researchers. The most extensive of these is that of Szkolnik (22, 23) who made observations over a 25-year period in New York. He noted during a span of 16 consecutive years that, almost without exception, some mature spores are released from leaves by the time of the green tip stage of apple tree growth; thereafter, the number of spores increased with time, reaching a peak about the late pink to early bloom stages; and then, spore output decreased until 95 per cent or more of the ascospore supply in the old leaves was exhausted, about 6 weeks after petal fall (Figs. 2, 3).

Ascospore productivity measurements of selected leaf samples may be useful in predicting relative expected doses in the air in the event of rains. In 1975, Gilpatrick made weekly observations on ascospore potential using the tower method. He noted a good correlation between the normal phenological development of host and fungus and spore potential (Table 4), thus confirming the observations of Brook (2) in New Zealand. In 1975, ascospore potentials were found to vary from one area to another in New York (Table 5). Ascospore productivity from selected leaves in an orchard will provide only a relative estimate of the actual inoculum pressure present. Total ascospore productivity in an orchard or apple growing area is dependent on the amount and timing of scab infections in the previous year, the number of infected leaves that overwinter, the weather from time of leaf fall to the end of primary scab in the following spring, and the activity of biological control agents. Knowledge of these factors is very limited.

#### **ASCOSPORE DOSE**

**METHODS**—The number of ascospores in the air has been determined with various types of spore traps (Table

Table 4.—Ascospore shooting potential for Geneva, New York in 1975.

	Relative			
Date	spore potential <sup>a</sup>	Apple growth stage		
April l	0	Dormant		
April 14	5	Dormant		
April 2l	11	Green tip		
April 27	340	1/4-inch Green		
May 5	9,000	3/4-inch Green		
May 12	5,000	Pre-pink		
May 21	69,000	Bloom		
June 2	5,000	Fruit set		
June 6	420	<del></del>		
June 16	100	<del></del>		
July 1	0			

a Twenty-five leaf discs, each from a different overwintered leaf, were submerged in water for 5 minutes and then processed in the shooting tower (6). Different leaves taken from beneath the same tree were processed on each date. The spore potential is the number of spores trapped from the 25 discs with a total surface area of  $125~{\rm cm}^2$ .

Table 5.—Ascospore potential for four different areas of New York in 1975 on three different dates.<sup>8</sup>

Gene	va	William	son	Gree	ce	Cha	azy
May 5	9,000	May 7	365	May 9	285	May 13	1,100
May 21	69,000	May 20	3,500	May 21 :	28,000	May 19	100,000
June 16	100	June 10	300	June 18	165	June 16	62,000

Number of spores obtained from 25 leaf discs with a total surface area of  $125~\mathrm{cm}^2$ .

6). A comparison of some of these was made by Sutton and Jones (19). The rotorod consists of spore collector rods which are rotated in the air by a constant speed DC motor.

Table 6.—Spore traps used for measurement of ascospore dose in air.

Trap	Reference	Comments
Filtration	Keitt and Jones (11)	
Rotorod	Metronics Associates, Inc. (14)	Measures total catch during
	Sutton and Jones (19)	operation.
		May operate only during
		wetting periods.
Hirst	Hirst and Stedman (7)	24-hour reading on a glass
	Brook (2)	slide.
	Miller and Waggoner (15)	
Burkard	Sutton and Jones (19)	7-day readings to the
	Gilpatrick (unpublished)	nearest half hour on a
		tape.
Wind vane	Sutton and Jones (19)	Measures total catch during
		operation.

This instrument is relatively inexpensive, highly efficient, simple to operate, and allows use of several replicate sample sites. It has been most useful in determining total spore dose during entire wetting periods. This instrument was utilized by Small (18) in western New York as early as 1958. His observations over several years served as a basis for grower recommendations for scab sprays. For critical studies of factors involved in spore release, the Burkard and Hirst traps are more useful than the rotorod in that spore catches can be defined to the nearest half hour and thus be related to other weather factors such as rainfall, wetting periods, and wind. The Hirst trap allows readings over a 24-hour period; the Burkard, over 7 days. High initial and maintenance costs of the latter two instruments limit their use as practical monitoring devices in grower advisory programs. Furthermore, it is unlikely that one instrument at an arbitrary site can furnish adequate information on spore populations in a single orchard, much less a region.

#### Effect of Environment on Ascospore Discharge

At any point in time in the spring in overwintering apple leaves on the ground, a certain number of asci within mature perithecia of *V. inaequalis* are capable of releasing ascospores following wetting of the leaves by rain. The actual number and duration of this release are dependent on several factors.

**PRERELEASE CONDITIONS**—Weather prior to discharge will determine the dryness of the leaf and the number of mature ascospores. The drier the leaf, probably the greater the amount of wetting that is needed for discharge; the more optimum the temperature, the greater the number of spores that will mature and be ready for discharge. Additional discussions of the effect of preconditioning on ascospore release have been made by Brook (3) and Hirst and Stedman (7,8,9).

RELEASE CONDITIONS-Leaf wetting by rain is an absolute requirement for significant ascospore release. Ascospores have been reported present in air following dews or in dry weather, but this mechanism probably plays no role in epidemics. The amount, duration, and intermittancy of rain will modify the spore release. Trace amounts of rain (0.2 mm or 0.008 in) may induce release (8), but greater amounts of rain and leaf wetting will usually result in release of much higher numbers of spores. Release may occur within a few seconds of leaf wetting; however, in the orchard, spore dose peaks are often observed about 2 hours after the start of wetting (8). In prolonged wetting, about 75 per cent of the discharge occurs within 3-6 hours; but, spores may be released intermittently and with secondary maxima, depending on environmental events and how quickly new spores are matured.

**Air temperature** probably plays some role in release (3). It has been reported that, as the freezing point is approached, the process is depressed or absent (8). The effect of differential temperatures, sudden temperature changes, or air/leaf temperature ratios is unknown.

**Time of day** may have a significant effect on ascospore release. Brook (3) observed that greater numbers of spores are discharged during daylight than during night darkness or during the afternoon than in the morning daylight hours. The reason for this is not understood but may be caused by the daily periodicity of temperature and light as they relate to spore maturation rather than a direct effect of light on the release mechanism. However, further clarification of the role of light in the ascospore discharge process would be useful for spray advisory services.

**Time of season.** Toward the end of the primary scab season, ascospores are not discharged as readily as in the earlier part of the season. This may be related to decomposition of leaves and perithecia as noted by Szkolnik (21) or to physical factors within the perithecia as they become emptied.

#### Effect of Environment On Ascospore Dosage

The number of spores in the air is dependent on ascospore productivity and factors that influence spore discharge as described above. Wind and rain intensity may be important. Ascospores are wind-borne; thus, their numbers at any one location in relation to the area of productivity will be determined by air mass movements and wind direction and velocity. Assumably, heavy rains will tend to wash many more spores from the air than will light rains.

#### DISCUSSION

During this century, extensive studies have been conducted on the ascosporic phase of the apple scab disease. Utilizing this information, Mills' wetting periods, and

fungicides, control programs have been developed that have provided a high economic level of control of this disease. Epidemiological studies of the post-Jones and Keitt period of the mid-1920's have developed insufficient new knowledge to allow for significant additional efficiencies in controlling this disease. Most progress has come from the development of more efficient fungicides rather than from epidemiological advances.

Our old and current technologies of assessing scab inoculum potential are useful predominately in providing information on the initiation, peaking, and termination of the primary scab season. Yet, such information as monitored by Szkolnik for over two decades can be useful. This weekly examination of ascosporic development in perithecia provided ongoing information on (a) the progressive differential shift in ascospore maturity and their level of depletion from leaves, and (b) the relative abundance of ascospore discharge from the leaves throughout the primary scab period. This information proved to be extremely valuable in the conduct of orchard spray research in New York and in the guidance of fruit growers in their spray programming, particularly at the beginning and end of the primary scab season.

Information on the real impact of measured inoculum potential (spore discharge and dose in air) in relation to scab potential, dosage response of fungicides, choice of fungicide, and timing of sprays is virtually non-existent. Thus, it is impossible and, indeed, hazardous at this time to interpret inoculum potential estimates in terms of practical spray recommendations except in broad and conservative ways. With a further understanding of inoculum potential in relation to disease potential, inoculum estimates could become an integral part of a more complex, computerized spray advisory service which would also utilize such parameters as fungicide mode of action, fungicide residues, tree growth, Mills' periods, and crop management practices (12). The prediction of inoculum discharge and dose will depend on weather, which is readily monitored, and improved weather forecasting. Unquestionably, at this time, sufficient information is available to allow the development of such inoculum potential prediction systems. Further research should emphasize the meaning of such predictions in relation to disease potential and measures for control.

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### ROLE OF CONIDIA OF VENTURIA INAEQUALIS IN THE EPIDEMIOLOGY OF APPLE SCAB

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The conidial stage of *Venturia inaequalis* (Cke.) Wint. (*Spilocea pomi* Fries) is largely responsible for the buildup of scab in the orchard in the late spring, summer, and fall following infection in the spring by ascospores. Recently, the importance of conidia, produced by overwintering infections on young shoots, as a primary inoculum source has been demonstrated in England (3, 7). This paper examines some of the factors influencing conidia production, dissemination, and viability.

#### **CONIDIA PRODUCTION**

Once penetration has occurred, the rate of lesion development is influenced primarily by temperature (11, 13, 17, 22) and leaf age (11, 15). Maximum lesion expansion occurs at 19 C with little expansion at 0 C or above 26 C. From 12 to 24 C lesions become macroscopic in 8 to 12 days. As leaves mature, the incubation period increases and after 30 to 55 days lesions develop on the surface of even the oldest leaves (11).

Conidia production begins before lesions are macroscopic (11). Sporulation occurs at temperatures ranging from 4 to 28 C, although maximum sporulation occurs from 16 to 20 C (20). Conidia are produced between 60 and 100 per cent relative humidity (RH) with 90 per cent RH optimum. Sporulation is reduced from optimum levels by about 50 per cent at 80 and 100 per cent RH (20). Day length does not affect sporulation; however, in continuous darkness sporulation is reduced by about 32 per cent (20).

Lesions expand to 6 to 10 mm diameter although lesion number, leaf age, and variety restrict their size. As lesions mature, sporulation in the center declines and some necrosis often appears. Although variety influences lesion development, lesion growth and spore production stops generally in 30 to 36 days, and lesions become necrotic or bronzed and reddish brown (15).

#### **CONIDIA VIABILITY**

The viability of conidia which are produced and remain attached to the conidiophores decreases with high temperature and/or RH. After 133 days at 0 per cent RH and 20 C, Louw (12) found that conidia germination was reduced from 73 to 52 per cent. Heuberger *et al.* (6) found no reduction in conidia viability after 120 hours at 20 C and 0 per cent RH, but at high RH viability decreased by about 50 per cent after 120 hours. At 35 or 38 C and 100 per cent RH, viability was reduced to 0 per cent after only 10 hours exposure; however, at 0 per cent RH viability was only reduced by about one-third after 120 hours. At 35 to 40 C, the time necessary to completely inhibit spore germination decreased with increasing RH (73 to 90%). Temperatures below 35 C with RH below 73 per cent had no effect on germination through 120 hours.

Heuberger et al. (6) and Connor and Heuberger (2) indicate that the effect of high RH and high temperature on conidia viability is reversible to some extent. They (6) found that after an exposure for 8 hours at 35 C and high RH, ap-

proximately 30 per cent of the conidia subsequently exposed for up to 72 hours at 20 C and low RH were viable, in contrast to 9 per cent viability for conidia receiving no treatment following exposure.

#### CONIDIA DISSEMINATION

Early work by Frey and Keitt (5) and Keitt and Jones(11) established the principle that rainfall is the most important agent of conidia dispersal. They found conidia abundant in drippings collected during rains from scabby apple trees and observed that on the addition of a drop of water to a lesion the conidiophores swell and conidia are immediately detached. The importance of rainfall as a dispersal mechanism for apple scab conidia has since been confirmed by numerous investigators. The largest quantities of conidia are disseminated by water during periods of prolonged rainy weather (1), when numbers of new lesions on the upper leaf surface are high, or when several days elapse between rains (19). Neither temperature nor wind velocity have any effect on the pattern of dissemination by rainfall (1).

The early work of Frey and Keitt (5) and Keitt and Jones (11) largely discounted the role of airborne conidia in the spread of apple scab. Using a volumetric spore trap, they caught only small numbers of conidia in the air and then only during windy, rainy weather. In the laboratory, excessive velocities were needed to remove conidia from sporulating lesions. Howitt and Evans (9), also working in the mid-1920's, caught conidia with greased slides placed at varying distances from an infected apple tree and concluded that airborne conidia were important in spreading disease from tree to tree in the orchard. However, their work received little attention, and Frey and Keitt's (5) conclusion that "no important dissemination of conidia is to be expected in the absence of water" essentially remained unchallenged for the next 35 years.

In 1960, Hirst and Stedman (8), using a volumetric spore trap, found conidia common in the air in the daytime during dry weather. They suggested the failure of earlier investigators to catch substantial numbers of spores may have been due to improper trap placement, imperfect trap design, or the difficulty of recognizing spores following treatment of the trapping surfaces. They found that although rainfall usually lowered the concentration of conidia in the air, occasionally spore numbers increased. Conidia catch was characterized by a diurnal periodicity with a peak in the early afternoon. This diurnal pattern was later confirmed by Sutton and Jones (19) who found the peak concentration to occur 2 to 3 hours later at their trapping site.

Sutton and Jones (19) using correlation and multiple regression analyses, investigated the significance of environmental factors on aerial dispersal. They found that dissemination of airborne conidia was generally associated with increasing temperature, sunshine, increasing wind speeds, low RH, and dry foliage. However, they note that the exact relationship of these factors to dispersal is com-

plex and far from clear. They suggest that rainfall probably has an initial effect of liberating spores through puff or splash dispersal and that continuing rain removes them from the air through its scrubbing effect. They feel the positive correlation of spore release and increased wind velocity may in part reflect mechanical spore removal by leaf abrasion and that the positive correlation with solar radiation may be spurious and confounded by a concomittant temperature increase and RH decrease.

Hirst and Stedman (8), noting that V. inaequalis conidia have been caught by spore traps in aircraft at 2,000 feet. postulated that airborne conidia are important epidemiologically in establishing disease in scab-free orchards in the fall, once spraying has ceased. It has also been postulated that airborne conidia might be important in increasing scab during periods without rainfall (19). Sutton and Jones (19) observed an essentially linear increase in scab during a 30-day period during the 1974 season in which there was only one rainy period of sufficient length to give infection. They suggest that part of this increase may have resulted from infection from airborne conidia deposited on leaves during the day, with germination and infection occurring at night when the leaves are wet from dew. They note that during this 30-day dry period, wetting from dew occurred on nine occasions of sufficient duration for infection to occur.

### GERMINATION AND SURVIVAL FOLLOWING DEPOSITION

Once conidia are deposited by rain or other means, germination and penetration is influenced by the temperature and duration of wetting. Germination occurs in the presence of free water from less than 1 C to 32 C with 19 C optimum (6, 10, 11, 12). Mills and LaPlante (13) indicated that infections from conidia became established in two-thirds the time of those for ascospores; however, Roosje (16, 17) and Moore (14), after a series of inoculation experiments, concluded that the minimum time necessary for infection by conidia was not shorter than the minimum period for ascospores and was probably longer. At 6.6 to 6.6 C, Roosje (16) found that the infection period was 2 to 10 hours longer than Mills indicated for ascospores, and Moore (14) found that conidia had to be wet for at least 1 hour longer than ascospores to establish infection.

Conidia which have germinated are very sensitive to drying (4). Roosje (16) noted that some partly germinated conidia can survive a dry period of 4 to 12½ hours between two leaf wetness periods, neither of which is sufficient to cause infection, but a dry period of 10 to 15½ hours between two wetting periods reduces attack to only 15 to 49 per cent of the numbers caused by continuous wetting. Moore (14) found that infection from conidia was sharply checked by a 24-hour dry period at threshold infection levels. He also found that wetting periods much longer than 18 hours were less favorable for infection than those shorter than 18 hours.

The survival of nongerminated conidia during dry

periods, following deposition on the leaf surface, has not been investigated in any depth, although they apparently respond similarly to those still attached to conidiophores. Weisman (21) found that conidia dried on slides were viable after periods of 6 to 10 weeks, and according to Jahn (10), conidia are not injured by desiccation on cover slips for periods up to 4 days and can be preserved in a viable state for several months at 0 C. Louw (12) observed at 0 per cent RH and temperatures from 0 to 19 C, viability was reduced by about 50 per cent in 22 days and that after storage for 17 days at 0 per cent RH and 26 or 30 C no conidia were viable. At 10 and 2 C and 70 to 100 per cent RH, Senger (18) found viability decreased by about one-half after 55 days and 75 per cent after 92 days.

#### CONCLUSION

The conidial stage of *V. inaequalis* has long been recognized as an important phase in the epidemiology of apple scab. Because of the immense capacity of lesions to produce spores, the potential exists for rapid scab buildup within an orchard. Successful control thus depends to a large extent on control of primary infection early in the season.

The importance of the conidial phase of V. inaequalis could take on an added meaning with the development of scab management programs. Apple scab monitoring systems currently used in pest management programs in the United States follow ascospore development and discharge in relation to infection periods and advise growers accordingly of the need to spray. If overwintering twig infections are important in other areas as in England (3.7). such monitoring systems could underestimate the importance of certain infection periods with low ascospore discharges, leading growers to erroneous management decisions. Therefore, the importance of the conidial stage as a source of primary inoculum should be carefully assessed in each area of the world as part of any management program. The spread of conidia from orchard to orchard through the air could also be important to a disease management program. In areas of the world where summer disease problems are slight, growers who do not have an early season scab problem often stretch spray intervals or reduce fungicide dose during the summer months. Conidia blown in from nearby orchards could establish infections in such orchards resulting in scab buildup. Extension personnel, agricultural fieldmen, and growers involved in pest management programs should be cognizant of this potential threat and follow a judicious scouting program through the summer months.

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### ORCHARD TEMPERATURE AND CONIDIAL INOCULUM OF THE APPLE SCAB FUNGUS

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Over the past 50 years, the epidemiology of apple scab has been investigated by many researchers. In several of these reports, it was noted that infection tended to occur less frequently during the latter part of the season, mainly during July and August. Many investigators felt that the high temperatures during this part of the scab season might be involved in the reduction of the disease.

Keitt and his co-workers obtained data on the effects of temperature on conidia germination and infection and on the disease severity on leaves and fruits. Mills developed a table for determining the level of infection likely to occur by using temperature and duration of leaf-wetness. Heuberger et al. studied the effect of temperature and relative humidity on the viability of conidia obtained from foliage lesions. They reported the loss of viability in late July and August for conidia from primary lesions but not of conidia from secondary lesions. This report and others indicate that the maximum temperature for germination inhibition was approximately 30 C. Many others have contributed information on the epidemiology of apple scab. In all of these studies, germination was the principal factor measured with few experiments conducted to obtain data on temperature and conidia production in the orchard.

During our studies on the epidemiology of apple scab, the effects of temperature on conidia production and viability from primary and secondary lesions were analyzed.

An experiment was conducted over a 13-week period in a four-row block of 18-year-old 'Staymared' apple trees. The plots were arranged in randomized complete blocks with four levels of disease control and four, four-tree replicates per disease level. The fungicide dosage per disease level was as follows: I, water; II, 16 oz Polyram 80W; III. 6 oz Cyprex 65W; IV, 6 oz Benlate 50W per 100 gallons of water. The sprays were applied on April 28, May 9 and 23, and June 9, 1975. The border rows between plots were sprayed at periodic intervals to keep the disease from developing in these trees. All data were collected at weekly intervals. Each week 10 infected leaves were collected at random from each tree. One lesion from each leaf was removed using a cork borer. Only young, active lesions were utilized. Spore suspensions were prepared by inverting the lesions in distilled water until all spores had been released as determined by microscopic examination. The number of spores per lesion was determined with a hemacytometer. From each spore suspension, 0.1 ml was placed in each of two depression slides. The depression slides were placed in a petri dish moist chamber and incubated in the dark at 20 C for 24 hours. Spore germination counts were made on at least 100 conidia per depression with germination considered to have occurred if the length of the germ tube exceeded one-half the diameter of the conidium and was normal in appearance.

Weather data were collected from instruments located in the orchard. The data were summarized by recording temperature and humidity at 2-hour intervals from hygrothermographs. Precipitation was accumulated for each 24-hour period. Leaf-wetness was recorded with a DeWitt Leaf Wetness meter. These data were analyzed by regression analysis.

Precipitation, relative humidity, and infection periods had very low correlation values with spore production, spore germination, and leaf infection. These low correlation values were probably due in part to the uniform distribution of these parameters throughout the experiment. Temperature was found to be correlated with spore production and germination. The temperature parameters obtained were maximum, minimum, median, and mean. The mean temperature was found to be the most significant. The daily mean temperature value was derived from 12 2-hour readings for each 24-hour period.

The mean temperatures increased consistantly from May 1 within a range of 13 to 16 C to a peak of 26 C on May 20. The mean temperature then declined to abjout 16 C on June 8, but by June 19, the mean temperature had increased to 26 C. For the remainder of June and August, the lower mean and maximum temperatures continued to increase. The range of mean temperatures was 20 to 28 C, and for the maximum temperatures, the range was 23 to 29 C for the period from mid-June to mid-August.

Observations of plotted data of mean temperatures, conidia production and germination, and viable inoculum indicated a lag period between mean temperature and its effect on inoculum. Mean temperatures were then accumulated over different time periods and regression values calculated against conidia production, germination, and viable inoculum. The most significant values were obtained with mean temperatures accumulated over a period of 9-13 days prior to each spore sampling date.

When conidia production was correlated with accumulated mean temperatures for disease control levels I, III, and IV, the r values were -0.789\*\*, -0.796\*\*, -0.717\*\*, and -0.663\*\*, respectively. Correlations of germination with accumulated mean temperatures had r values of -0.681\*, -0.548, -0.684\*\*, and -0.410 for disease control levels, I, II, III, and IV, respectively. When the viable inoculum was correlated with accumulated mean temperature, the r values were -0.774\*\*, -0.778\*\*, -0.735\*\*, and -0.665\* for disease control levels I, II, III, and IV, respectively.

These r values indicate that the most significant effects of the accumulated mean temperature 9-13 days prior to sampling are on sporulation and viable inoculum. There are also significant effects on conidia germination in two of the disease control levels. The effect of accumulated mean temperature 9-13 days prior to spore sampling on conidia

production is supported by last year's experiment where there was a significant correlation in four of the six treatments.

In 1974, an experiment was conducted to measure the eradicative effect of various fungicides on established apple scab foliage lesions. With the information obtained from the 1975 experiment, it was decided to measure this temperature effect using the 1974 data. Although the 1974 experiment was not designed to specifically measure the temperature effect, there was enough essential data to obtain an indication as to its effect on spore production and germination. Accumulated mean temperatures 9-13 days prior to collection had the best correlation with spore production. In four of the six treatments, the correlation values were significant at the 0.5 per cent level or higher when accumulated mean temperature was compared with spore production. Only one of the six treatments was significant with germination.

Many observers have noted the reduced amount of disease which tends to occur during July and August. The reason generally suggested for this reduction has been the high temperatures occurring during this period. Conidia germination was considered to be the primary target of these high temperatures and therefore most of the studies were conducted on conidia germination. Another possible explanation suggested for the reduced disease incidence during July and August was the age of the lesions sampled. This can be an important factor if only primary lesions are sampled during these months or if only old lesions are selected.

The data obtained during these experiments indicate that the principal effect of high temperatures during July and August was on conidia production with a less significant effect on conidia germination. The resulting effect would be a reduction in disease incidence.

These findings suggest that the use of accumulated mean temperature 9-13 days prior to sampling or conidia production may be utilized as a tool for predicting when the inoculum level may be reduced and therefore when fungicide usage for scab control may be reduced. Only further information will demonstrate the effectiveness of this tool in an integrated pest management program. We are currently obtaining further information on temperature and other environmental and biological factors affecting apple scab.

### RELATIVE SUSCEPTIBILITY TO SCAB AND PRODUCTION OF CONIDIA AMONG 30 APPLE VARIETIES

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Over a span of many decades various investigators have reported on the relative susceptibility of apple varieties to apple scab caused by *Venturia inaequalis* (Cke.) Wint. These reports have been largely sporadic involving few varieties in one apple growing area or another and usually without definite experimentation designed to factually ascertain the relative susceptibility among apple varieties even in a given area. Comparisons have been made among varieties based on field observations under varying conditions of weather and disease pressures of different years. Often, distinctions have not been made of relative susceptibility between fruit and vegetative tissues of the same variety in relationship to the determined pressures of the primary and secondary phases of scab.

Even with cultivars like Red Delicious and Golden Delicious, grown commercially in different regions of the country, there has been no common denominator of technique for assessing relative susceptibility to scab. Observers in each region devised their own subjective assessment values which in one region may not have coincided with those of another. Consequently, it has been next to impossible to prepare a factual composite summation of many apple varieties grown in the United States. To assemble such information would first require considerable new observations and experimentation under well coordinated and defined techniques and categories used by investigators of all apple growing areas.

The author has studied 30 apple varieties for their relative susceptibility to foliar infection under like conditions. Initial comprehensive studies were made in the greenhouse under controlled conditions of tree culture, conidial inoculation, and infection. Later, this work was expanded to the exposure of greenhouse grown trees to natural ascosporic inoculation and infection in the orchard. Scab susceptibility was based on number of lesions per leaf for different age leaves, lesion types, and the production of conidia from lesions.

#### **METHODS**

Commercially acquired apple nursery stock trees were planted and maintained in 7-inch clay pots in a substrate mixture of one part good field soil, one part sand, and two parts peat moss. The trees were grown in the greenhouse with normal maintenance with a weekly application of a complete soluble fertilizer including minor elements. Five trees of each apple variety were used for each greenhouse or field test.

#### **Greenhouse Tests:**

At the beginning of each experiment, a small piece of cotton string was tied to the petiole of the youngest unfolded apical leaf (designated "S") on all trees to later facilitate the recording of data for specific leaves. The conidial inoculum was secured from lesions of recently infected greenhouse grown trees. The conidia were washed off the leaves into a beaker with distilled water delivered from an atomizer at 30 psi. Standardization of spore suspension was through dilution with distilled water to a concentration of 50,000 conidia/ml. The inoculum was carefully applied to the leaves by atomizer to achieve a deposition of a uniform pattern of droplets (without runoff) which are more conducive to individual lesion development which allows accurate lesion counts. The inoculum was allowed to dry on the trees so as not to disrupt the droplets by handling. The trees were then given an infection period in moist chambers at 100 per cent relative humidity and 20 C for 30 to 36 hours, and then returned to the greenhouse for completion of the incubation period. Visible infection often was first noted about 8 or 9 days after inoculation. Scab assessments were made about 2 to 3 weeks after inoculation by counting the lesions per leaf for the "S" leaf and successively older leaves on each tree. Lesion types were also recorded.

Conidial production was determined by carefully collecting specific comparable aged leaves of known lesion count. The collector's hands were washed after processing each variety to prevent accidental "cross contamination." In the laboratory, the spores were washed off the leaves with water using an atomizer under 30 psi pressure. Water was added to bring the spore suspension up to a standard volume. Samples from well agitated suspensions were counted in a hemocytometer. In this way, the number of spores per variety sample was determined and from this value the number of spores produced per lesion was calculated

#### Field Tests:

The potted vegetative trees were transferred from storage to the greenhouse about the end of March and allowed to grow as single shoots. When they had developed about 10 leaves, the trees were set adjacent to an abandoned McIntosh orchard at the beginning of the primary scab period at about the green tip stage of fruit bud development of orchard trees. A string tied to the smallest unfolded leaf (designated "S") on every tree identified the growth of shoots at that time. The trees were left there for about 6 weeks covering most of the primary scab period. Overwintered apple leaves in the orchard were monitored weekly for scab ascospore maturity and release to determine the inoculum potential. Records were taken of temperature and duration of wetting periods to determine actual natural infection periods. When an infection period occurred, a small piece of cotton string was tied to the petiole of the voungest unfolded apical leaf for reference later in determining which specific leaves were susceptible during that infection period. At the end of this 6-week exposure to natural infection conditions, the trees were returned to the greenhouse at a temperature of about 22 C. After allowing 2-3 weeks for development of all naturallyoccurring infections, data were taken on lesions per leaf, lesion types, and spore production as described earlier.

#### **Lesion Types:**

The following categories of scab lesion types on the leaves in progressive order of severify were employed throughout the greenhouse and field studies:

- A. Hypersensitive response "lesions" manifested as small depressions or dimples without chlorosis or necrosis.
- B. Small, angular, chlorotic lesions.
- C. Medium sized (approximately 4-6 mm), mostly round, chlorotic lesions.
- D. Medium sized and larger lesions, mostly round, and varying degrees of olive green to brown in color indicative of sporulation. Some lesions may be necrotic.

#### **RESULTS AND DISCUSSION**

Most of the results presented are from the investigations in the greenhouse which were more extensive than those on the same 30 cultivars under orchard conditions. In the latter case, the research was initiated at a later date and is still incomplete. Tests in 1976 were hampered by a hailstorm which severely damaged the foliage of the test trees.

In the relatively uniform and protected environment of the greenhouse and with good maintenance of watering and fertilizing, the single-shooted trees grow vigorously. Shoots often reach 4 to 5 feet before termination of growth; however, the height differs among varieties. This growth was probably three to four times that of vegetative shoots (terminals) normally found on established bearing orchard trees. Greenhouse grown shoots produce about four new leaves a week compared with an average of two leaves or

less on orchard trees.

The leaves of growing terminals in the field attain full size and maturity and become resistant to apple scab infection much sooner than do those on greenhouse trees. This may be due to differences in physiology or thickness and nature of the cuticle and wax layer of field trees growing under more natural weather conditions of greater extremes than do greenhouse grown trees. This appears evident when considering the number of leaves that can be infected on the shoot during a single infection period. In the orchard, scab from one infection may occur on only the two or three youngest leaves whereas in the greenhouse eight or more leaves on the more susceptible varieties may become infected (Table 2).

#### **Greenhouse Tests:**

In the greenhouse tests, the youngest leaf (S leaf) was most susceptible to the scab disease and susceptibility diminished with each consecutive older leaf on the shoot (Table 1). Although fewer lesions developed on the youngest leaves than on the nearest older leaves, when lesion count was equated to leaf size at time of inoculation, the youngest leaf developed the highest number of lesions per cm² with counts diminishing on each older successive leaf.

Although the severity of infection decreases with leaf age, the number of leaves on greenhouse growing trees that became infected during one infection period varied with the variety. On highly susceptible varieties like Mutsu, Niagara, and McIntosh, eight or more leaves developed scab (Table 2). Even though the youngest leaves on varieties like Delicious and Empire became readily infected with a high lesion count, the severity of scab dropped off sharply after the fourth leaf. All the young leaves of the unnamed variety COOP #3 were highly resistant to infection, with practically no infection occurring beyond the fourth leaf.

Table 1.—Relative susceptibility of successive apple leaves of increasing age to apple scab as indicated by lesions per leaf and lesions per unit area based on leaf size at the time of inoculation and infection.

Apple leaf position	Scab l	esions per cm <sup>2</sup> a	Leaf <sub>2</sub> area cm <sup>2</sup> a
<b>~</b> -	<del></del> 36	7.1	5
<b>—</b>	56	4.5	13
•	59	2.4	25
<b>&gt;</b> -	44	1.1	39
	24	0.5	50
<b>→</b> -	12	0.2	58
	6	0.1	61
,			

a Based on leaf size at inoculation date.

Table 2.—The level of apple scab infection on leaves of different age on apple varieties of varying susceptibility to scab.

		at	Cab :	lesior erent	ns per age o	lea: n sho	f oot <sup>a</sup>	
Variety	s	-1	-2	-3	-4	<b>-</b> 5	-6	-7
Mutsu	37	73	84	70	54	37	20	18
Niagara	25	43	60	58	43	35	23	17
Geneva McIntosh	25	57	75	56	37	18	12	4
NY 44408-5	29	41	43	34	25	20	12	10
Golden Delicious	41	53	61	45	25	17	9	8
Rome Beauty	46	57	56	44	26	13	8	1
Cortland	28	61	76	48	27	11	4	1
Jonadel	25	30	33	24	11	7	3	0
Empire	45	73	57	23	4	3	0.1	0.5
Gardner Delicious	45	57	49	22	7	3	0	0
NY 58553-1	4	13	16	10	1	0	0	0
COOP #3	2	3	5	1	0.2	0	0	0

Average of 7 tests of one inoculation and infection.

#### Lesions per leaf

Table 3 summarized data from at least seven greenhouse tests on the average number of lesions per leaf for 30 apple varieties based on lesions counts of the second, third, and fourth youngest leaves on the shoot at the time of inoculation. Infection levels ranged from 87 lesions per leaf for NY 18491 to only three lesions for COOP #3.

Table 3.—Relative susceptibility of 30 apple varieties to apple scab (*Venturia inaequalis* [Cke.] Wint.) under greenhouse and field conditions as gauged by lesions per leaf, lesion type, and spore production.

	Scab lesions per leaf		lesion ype b		per lesion
Variety	GH <sup>a</sup>	GH	Field	GH	Field
NY 18491	. 87	Cd	Cb	6	16
Lodi	. 79	Dc	DC	94	200
Mutsu	. 76	D	D	158	262
Idared	. 72	Dc	Dc	103	192
Macoun	. 66	DC	CD	111	267
Milton	. 65	CD	Dc	44	121
Geneva McIntosh	. 63	Ď	D	144	421
Cortland	. 62	D	Dс	163	205
Boller McIntosh	. 62	D	D	126	183
Vellington	. 60	Cd	CD	56	168
Jonagold(NY 43013-1)	56	D	D	171	398
Spigold	. 54	D	D	185	514
Niagara	. 54	D	DC	158	355
Golden Delicious	. 53	Dc	D	144	263
Rome Beauty	. 52	D	D	181	252
/PI-5	. 52	Cd	CD	41	88
Empire	. 51	D	D	93	206
NY 55158-2	. 51	В	В	2	2
Spartan	. 49	D	D	96	204
Monroe	. 48	Dc	D	63	373
Vayne	47	Dc	D	112	231
NY 55140-9	46	В	В	0.4	16
TY 44428-3	46	Dc	Dc	80	169
Gardner Delicious .	4.3	D	D	105	176
IY 44408-5	. 39	Dc	D	89	344
riscilla (COOP #4) .	. 31	В	В	0.02	0
Jonadel	29	CD	DC	23	270
OOP #1	15	Bc	В	2	Ö
ry 58553-1	13	A	A	0.5	õ
000P #3	. 3	AB	Ab	5	0

a Average of 7 greenhouse tests.

#### Lesion types

The majority of varieties produced foliar lesions of Type "D," the typical sporulating type. Although the varieties with the highest number of lesions per leaf tended to have lesions of Type "D," no such general conclusion could be made. For instance, Empire with 51 lesions per leaf, had "D" type lesions whereas NY 55158-2, also with 51 lesions per leaf had "B" type lesions. Other exceptions are found in Table 3.

#### Spore production

The number of lesions per leaf cannot be relied upon alone as an indicator of varietal susceptibility to scab without the added important factors of lesion type and spore production per lesion. For instance, the unnamed variety NY 18491 had the highest number of lesions per leaf in greenhouse tests, yet the lesions were largely chlorotic "C" type with an average production of 6,000 spores per lesion compared to Mutsu which produced 158,000 spores per lesion of the "D" type (Table 3). The NY 55158-2 variety had 51 lesions per leaf which were predominantly "B" type and producing scarcely 2,000 spores per lesion. Only about 20 spores per lesion were produced on the Priscilla (COOP #4) variety with "B" type lesions.

#### Field Tests:

The greenhouse grown trees in the harsh environment of the orchard grew much differently than in the controlled environment of the greenhouse. Existing leaves soon after the trees were set in the field developed a red to purple color. With the relatively lower prevailing temperatures in the field, the trees grew more slowly; leaves were appreciably smaller and attained maturity and tolerance to scab earlier, compared to greenhouse trees. Unlike in the greenhouse where single inoculation and infection of trees can be controlled and monitored for severity on specific leaves on the shoot, trees in the orchard are subjected to multiple infection periods of differing severity. Thus, it is not readily possible to separate the impact of different infection periods on the relative susceptibility of leaves at specific positions on the shoot. Assessment of relative varietal susceptibility as determined by leaf age (lesions per leaf area basis) will be deferred until more data becomes available.

#### **Lesion Type**

Observations made so far on scab leaf lesion type reveal a very close parallel between greenhouse and field (Table 3). Thus, the susceptible varieties like McIntosh, Mutsu, and Rome Beauty have "D" type lesions in both environments; the tolerant varieties, Priscilla (COOP #4) and the unnamed NY 55140-9, have predominantly "B" lesions; and the highly tolerant (resistant) NY 58553-1 and COOP #3 have "A" and "AB" lesions, respectively.

It is possible for a given apple variety to have more than one predominant lesion type from any infection, whether it

S,-1,-2,etc. = Smallest unfolded apical leaf at time of inoculation and successive older leaves on shoot.

b With dual letter designation, first lesion type predominates; small letter indicates type for about 25% of the lesions on the leaves. GH (Greenhouse): Controlled conidial inoculation and infection. Field: Natural ascosporic inoculation and infection.

be field ascosporic or greenhouse conidial infection. In a number of instances, a variety with predominantly "C" type lesions can have a varying number of "D" and "B" type lesions also, even on the same leaf. Leaf age and condition and conditions of an infection period may affect the lesion type.

#### **Spore Production**

With limited results on spore counts on leaves from field infected leaves, there is a reasonably close parallel among varieties on productivity of conidia by lesions of different types (Table 3). The greatest level of conidial production occurred mostly on "D" type lesions with considerably fewer on "C" type. There were extremely few to no spores produced on "B" lesions and none from "A" lesions. The

comparative productivity of conidia from leaf lesions among the 30 apple varieties infected in the field bears a close similarity to that of the same varieties infected in the greenhouse. The higher spore count per lesion on field trees is mostly a factor of larger lesions and fewer lesions per leaf occurring on field trees stemming from lesser inoculum and infection condition pressures compared to exposure of trees in the greenhouse. In the assessment of apple varieties for tolerance to scab, spore productivity is a key criterion to consider because of the impact on secondary spread of apple scab.

Evidently, several varieties tested which have emerged from the apple breeding programs of New York, Indiana, New Jersey, and Virginia possess a high degree of tolerance to scab which, under most field conditions, may constitute virtual immunity.

#### EPIDEMIOLOGY OF PEAR SCAB IN ISRAEL

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Pear scab, caused by *Venturia pirina*, is the most damaging pest of pear in Israel. In 1944, scab was reported to exist on some of the pear cultivars grown at that time in Israel (4). Yet, only in 1962, scab was found for the first time on a few fruits of the Spadona cultivar, which was introduced into Israel from Italy in the 1940's and is the principal pear cultivar.

In several areas of the world, *V. pirina* has exhibited pathotypic variability (2, 8, 10). Shabi *et al.* (6) isolated five races of *V. pirina* from cultivated (*Pyrus communis*) and wild pears (*Pyrus syriaca*). Race 2 is the incitant of scab epidemics in commercial orchards. Races 1, 3, and 4 infect pear cultivars which have no commercial value, and race 5 infects the wild pear, *P. syriaca*. Since pear scab became widespread in orchards, many farmers have suffered heavy losses even though fungicides were applied for scab control. When adequate control measures were not used, the entire crop could be destroyed by fruit infection. Failures in disease control were primarily caused by inadquate applications of fungicides, but pathogen tolerance to fungicides has been found also (7).

The weather conditions in Israel prevailing after bud burst are favorable for scab epidemics in most years, even though there are few spring rains and no summer rains. Measurements of the phenological development of *P. syriaca* and some *P. communis* cultivars were made during the last 10 years and compared with the development of *V. pirina* and pear scab. The influence of spring rains, dew, and irrigation on epidemics of pear scab was also studied.

In early spring, V. pirina inoculum is present in previously infected orchards; the pathogen overwinters in infected twigs on the trees and in leaves on the orchard floor (1, 2). At the end of February, susceptible tissue of P. syriaca is always exposed to V. pirina inoculum; green tissue of the Spadona cultivar is not present until mid-March (Table 1). Therefore, scab symptoms were observed earlier on the P. syriaca host than on the Spadona host. Such relationships between host phenology and first scab infection were observed also on other pear cultivars. Scab symptoms could be found only in late April on the Amanlis cultivar, which was the latest to break dormancy (end of Marchearly April). In most years, rainy days occurred during each of the periods: mid-March, end of March, and early April (Table 1). Therefore, after the bud burst stage of the P. communis cultivars there were always enough rainy days for scab epidemics to begin.

During March and April, the temperature in Israel is mild. The maximum and minimum temperatures which were recorded during three springs at Nave Yaar in the Jezreel valley, which is a major pear growing area, are summarized in Table 2. During most days, the temperature was in the range of 5-25 C which was found to be suitable for pear scab infection (6, 9). If Mills' tables (3) can be applied to pear scab, 8 to 16 hours of wetness are sufficient for infection to be initiated under the temperature conditions prevailing in March and April.

In the spring of 1976, ambient temperatures and the duration of leaf wetness following rain were recorded on a

Table 1.—Phenological stages of two pear hosts in Israel during 1967-1976.<sup>8</sup>

						N	umber (	of year	s						
	Febr	uary			Mar	rch					A	pril			
Host		20-28			-10	11-	-20	21-	-31	1-	-10	11-	-20	21	-30
phenology	PS <sup>b</sup>	PCC	PS	PC	PS	PC	PS	PC	PS	PC	PS	PC	PS	PC	
Dormant		10		4											
Bud burst	3	0		6		4									
Green cluster white bud	6	0	7	0	2	4		3							
Bloom	1	0	2	0	6	2	3	6		3					
Fruit set	0	0	1	0	2	0	7	1	10	7	10	10	10	10	
First scab symptoms	0	0	1	0	2	0	6	4	1	5	0	0	0	1	
Total years with rainy days	1	.0		6		8		8		8		3		3	
Total number of rainy days	5	2	ĵ	L8	3	33	2	21	]	L9		9		3	

<sup>&</sup>lt;sup>a</sup>From bud burst to fruit set.

Table 2.—Temperatures in Nave Yaar during a March 1-April 30, 1974-1976.

			Number	of Days		
		Minimum		avar-	Maximum	
С	1974	1975	1976	1974	1975	1976
< 5	1	2	5	0	0	0
5 - 10	28	29	25	0	0	0
10 - 15	28	23	28	4	2	4
15 - 20	4	7	2	19	16	29
20 - 25	0	0	1	26	21	21
>25	0	0	0	12	22	7

modified 7-day thermograph, through the use of a battery-powered electronic amplifier. The sensors were made from two metal clips and placed on each of four green clusters or young leaves of a pear tree. Whenever rain water was present on the clusters or leaves, the electronic circuit was completed and wetness duration was recorded. For this purpose, an additional pen arm was built in the thermograph; it recorded the wetness duration on the same chart that temperature was recorded. By knowing the length of wet periods, temperatures, and the growth stage of the host, three infection periods were determined from March 19, 1976 (bud burst of Spadona cultivar) until the end of May (Table 3).

Table 3.—Infection periods of pear scab, March 19-May 31, 1976, Bet Dagan, Israel.

	Wetness duration	Temperature during wetness			
Date	(hours) a	Minimum	Maximum	Host phenology	
March 20	13	14	20	Bud burst	
April 4-5	16	13	17	Bloom petal ful	
April 5-6	14	12	17	Bloom petal ful	

<sup>&</sup>lt;sup>a</sup>Film of water on leaves during and after rain.

Table 4 summarizes the dates of the first spray applications for scab control, first scab symptoms, and the rain which initiated the first scab infections in orchards where fungicide tests were carried out, in the last 10 years. Scab symptoms were observed 12—19 days after the rainy day that seemed to initiate the first scab infections in these years. In experiments, conducted under controlled conditions in growth chambers (6), the incubation period of the disease was determined. Under the most favorable conditions (24 hours in a moist chamber at 20 C and then in growth chamber at 20 C), the shortest incubation period on the youngest leaves was 9 days. Therefore, under the natural conditions which prevailed in the orchards; i.e., night temperatures during March usually under 15 C, incubation periods of 12-19 days could be expected; the estimates of the first rain-initiated infection periods seem to be reasonable ones.

bPyrus syriaca.

<sup>&</sup>lt;sup>c</sup>Pyrus communis 'Spadona'.

Table 4.—Dates in pear orchard in which scab control tests were conducted during 1967-1976.

Year	First spray <sup>a</sup>	Rain initiated first scab infection period	First scab symptoms	Days of incubation
1967	March 20	April 4	April 23	1.9
1968	March 8	March 27	April 8	12
1969	March 13	March 19	March 31	12
1970	March 8	March 18	April 1	14
1971	March 10	March 18	March 31	13
1972	March 13	March 22	April 6	15
1973	March 8	March 7	March 25	18
1974	March 7	March 14	March 31	17
1.975	March 19	March 18	April 3	1.6
1976	March 15	March 20	April 2	13

and bud burst stage of Spadona cultivar.

In addition to the rain which initiates spring scab infections, it is also important to consider the role of dew and irrigation occurring late in the spring. Pear orchards in Israel are irrigated regularly after mid-April, when rain is scarce. Irrigation water is generally applied from under the tree canopy. It was found that leaves collected from the orchard floor during April, May, and June had high ascospore potential. (E. Shabi, unpublished data). Therefore, it can be suggested that irrigation causes the ejection of ascospores from perithecia on the orchard floor during the late spring and early summer months. Moisture on the plant surface, necessary for infection, is likely to be supplied by dew. During the spring, summer, and fall it is common to have nights with 6-10 hours of dew (5) and temperatures in the optimal range for infection by V. pirina. Thus, it is quite possible for scab epidemics to continue to develop during May and June even in absence of rain and incite late infections on leaves and fruits.

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# HANDLING THE APPLE SCAB ORGANISM IN LABORATORY AND GREENHOUSE

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Venturia inaequalis (Cke.) Wint., the causal agent of apple scab, has been used in classical studies on the genetics of microorganisms by Keitt and his co-workers at Wisconsin (1) and in programs for breeding for scab resistance in apple (4). The basic methods used in handing the organism in these and other major scab testing laboratories in the United States and Canada had their

origins in the work of Keitt and Palmiter (2) and Nusbaum and Keitt (3).

The following techniques are currently being used in the laboratory and greenhouse in our apple scab program at Purdue. They are simple, efficient, allow the production of a large quantity of inoculum quickly, and are adaptable to the inoculation of single plants with single isolates.

#### **TECHNIQUES**

Single Spore Isolation.—Invert a test tube with 2.5 ml sterile water over a freshly sporulating scab lesion on an apple leaf. Pour this suspension over the surface of 2 per cent strained water agar in a petri dish so that a water film just covers the surface. Incubate at 18 C for 6 hours. Drain the excess water from the surface. For the isolation procedure, two or three germinating spores per microscope field (45x) are preferred. Isolation is facilitated with a binocular dissecting scope of low magnification (45 to 90x). Sterilize a transfer needle tip (flat tip 1 mm or smaller) by heating. Cut along both sides of spore and germ tube, with the cuts intersecting above the germ tube apex. Turn the needle so that the broad side is horizontal. Cut behind the spore and lift out. Transfer the spore to a potatodextrose agar (PDA) slant in a capped tube (110 x 20 mm). Incubate the culture at 18 C until growth reaches the edges of the slant. Sufficient growth is usually obtained by 3 weeks for retransfer to tubes or wick culture. Cultures may be held in storage for an extended period under sterile mineral oil which has previously been autoclaved for 2 hours and cooled. The oil should cover the upper rim of the PDA slant. Cultures under oil in capped vials will remain viable and pathogenic for over 10 years at 1-4 C. Cultures can be maintained on PDA slants in capped vials for a year or more at 1-4 C without oil.

Culture Activation.—Using a glass or plastic smooth-tipped rod, spread a cheesecloth pad (50 x 100 mm) along one side of a 220-m! prescription bottle (Moderne Ovals, Foster Forbes Glass Company, Marion, Indiana). Pipette 25 ml of 4 per cent malt extract medium in each bottle. As the pad becomes moist, it will adhere to the side of the bottle. Loosely cap the bottle and sterilize. After cooling the medium, use a broad-tipped (2 mm) transfer needle to cut a plug of mycelium and agar from the edge of a freshly growing PDA slant culture and spread this in a thin line on the cheesecloth pad about 1 cm above the liquid surface when the bottle is placed on its narrow side. Incubate at 18 C for 2 weeks. The mycelium will grow down to the medium, sporulating at the interface.

**Inoculum Buildup.**—Prepare 220-ml prescription bottles with cheesecloth pads as described above. Place cheesecloth strainers (100 x 100 mm) over the tops of empty 125-ml Erlenmeyer flasks and secure them with rubber bands. Invert a 50-ml beaker over the top of each flask. Sterilize the bottles, flasks, 125-ml Erlenmeyer flasks containing 75 to 100 ml distilled water, and 10-ml pipettes (one for each isolate used). Allow the medium in the bottles and water flasks to cool before using.

Carefully pour off the medium from the scab culture bottle prepared 14 days earlier (preceding section). Rinse the bottom and narrow side of the source bottle with sterile distilled water. Pour this water off and then add 20 ml of sterile distilled water to the source bottle. Cap and agitate the bottle until a majority of spores are dislodged from the cheesecloth pad. Heat the opening of the bottle by flaming,

lift the inverted beaker from the empty flask, and strain the spore suspension through the cheesecloth into the flask. Remove the strainer and replace the inverted beaker. Pipette 2 ml of this spore suspension into each of five bottles of 4 per cent malt extract medium and then place each bottle on its flat side with the cheesecloth wick down. Incubate at 18 C for 4 days and then turn the bottle on its narrow side so that the cheesecloth wick dips into the medium. Incubate an additional 10 days at 18 C, after which the conidia are ready to harvest.

**Inoculum Preparation.**—Carefully pour off the liquid medium from the inoculum bottle. Rinse the sides and bottom of the bottle with tap water. Add 80 ml tap water to the bottle. Agitate vigorously, and then pour this suspension into the next inoculum bottle and repeat the agitation. Each 80 ml of water may be passed through eight or nine bottles. When the spore concentration is sufficient (2 x 10° per ml), strain the suspension through cheesecloth into 2,000-ml flask. Continue with other bottles until sufficient inoculum is obtained.

**Inoculation of Plants.**—Young seedlings can first be inoculated when the primary leaf is fully developed. Older seedlings and clonal material need actively growing tips for successful inoculation and symptom development.

When using a large group of plants, young seedlings are planted individually in 12-unit plastic multipots (Vaughn's Seed Company, Chicago, Illinois). Wooden frames are placed over the benches and covered with moist, bleached muslin. Portable humidifiers are operated on each side of the bench. The plants are inoculated with a spore suspension using an electric paint sprayer (Burgess Airless Paint Sprayer, Burgess Vibrocrafters, Inc., Grayslake, Illinois). Plants are held at 18-20 C for 40 hours under the muslin cover. The muslin is then removed and the humidifiers operated for an additional 48 hours. Maximum symptom development occurs after 8-10 days.

Small group or individual plant inoculations are made in a 6 mm clear plexiglass chamber (57 cm wide, 40 cm deep, and 53 cm high). The lid is also made of plexiglass and fits snugly on the chamber. The sides of the chamber are lined with Whatman #1 filter paper sheets. When moistened, these adhere to the walls. About 2.5 cm of water is placed in the bottom of the chamber. By double-decking the pots, 20, 12-cm pots can be included in each chamber. The plants are inoculated individually by means of a #15 De Vilbiss adjustable tip atomizer, and then incubated at 18-20 C in the closed chamber for 40 hours and then left in the chamber without its cover for an additional 24 hours. Scab symptoms develop within 8 to 14 days.

Maintaining Identity of Cultures.—Each isolate is numbered and its origin, host variety, date of isolation, type and number of spore, and race designation, if known, recorded. Each single spore is identified by isolate and spore number.

In bulk inoculations, from 20 to 40 individually grown cultures are used, and the inoculum is prepared just prior to application. These isolates are obtained from the wild each year from as broad a geographical area as possible including isolates of all known races.

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### **CURRENT STATUS OF APPLE SCAB RESISTANT VARIETIES**

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The program for the development of apple varieties resistant to scab, caused by Venturia inaequalis (Cke.) Wint., was initiated in 1945 by Dr. L. F. Hough, University of Illinois, and Dr. J. R. Shay, Purdue University. Since then, a formal cooperative program designated as PRI has been in effect between Purdue, Rutgers University, and the University of Illinois. In North America, other centers engaged in similar projects are at the New York Experiment Station, Geneva, New York, and the Canadian Research Centers at St. Jean, Quebec, and Kentville, Nova Scotia. The St. Jean project was originally located at Ottawa, Canada. Many other states and countries have cooperated informally with the PRI group. This cooperation primarily involved testing advanced selections. One station, the Penisular Experiment Station at Sturgeon Bay, Wisconsin, has, in addition, made its facilities available for part of our controlled cross-

With the PRI group, the following procedure is used in the development and release of new selections: Controlled collinated seeds obtained from crosses made at the three universities are collected in the Botany and Plant Pathology Department at Purdue. After stratification and germination, the young seedlings are inoculated with the scab organism. The susceptibles are discarded, and the resistant survivors are planted in fruiting blocks on one of the University farms. Fruiting occurs within 4 to 7 years. Those seedlings exhibiting desirable pomological characters as well as partial or total resistance to other pathogens are propagated on other rootstocks and planted in our holding block. Here they are held for future evaluation as potential parents or varieties. Those showing varietal potential are included in our Co-op series and are placed with selected growers. Here they are evaluated under commercial orchard conditions. Those having the characteristics to compete successfully are patented, named, and released to selected nurseries.

From the PRI program, four selections have been named. These are Prima (2), Priscilla (7), and Sir Prize (5), released for use in the United States, and Priam (3), released for use in the Loire Valley in France. These four varieties are resistant to scab and show varying levels of resistance to other pathogens. In addition to these varieties, 15 other selections have been placed in the Co-op series and 4 of these are presently being patented.

Two other scab resistant varieties have been released by apple breeding centers in Canada. These are Nova Easygro from the Kentville station (1) and Macfree from the Ottawa program (4).

Prima is a high quality dessert apple that ripens about 3 weeks before Jonathan, which it resembles to some extent. It is favored by reduced fertility, tending to become overly large with reduced color and Jonathan type spots under excessive nitrogen. The tree is somewhat dwarfed with wide angle crotches and requires little pruning. The leaves are resistant to mildew, while the fruit is somewhat susceptible under favorable conditions. It has a storage life of 4 to 8 weeks.

Priscilla matures about 2 weeks before Delicious. It is a fine fleshed, high quality dessert apple, reaching its peak in quality around Christmas. The fruits tend to be small with a heavy crop and may require thinning. The finish and color are excellent when grown correctly. The foliage is moderately susceptible to mildew, resistant to cedar rust, and has as much resistance to fireblight as Delicious. The tree is upright and needs to be headed back when grown on standard rootstock.

Sir Prize matures with or just ahead of Golden Delicious. The fruits are large, golden colored, with fine finish. The tree produced an annual crop with self thinning so that it does not tend to over produce. The mature fruit cells are thin walled and tender so that it has a tendency to bruise. Thus, it cannot be handled over a commercial grader. The variety

has high dessert quality, reaching its peak in late February and March. The fruits are non-shriveling with a refrigerated storage life up to a year. Because of the bruising characteristics, the variety should not be considered for commercial plantings but as a backyard or garden variety.

The variety Priam was originally sent to France as a numbered selection to be used as a parent in their scab resistance breeding program. It produces large, highly colored, attractive fruits under our conditions. It is, however, highly acidic and was judged not suited to American tastes. It proved to be well adapted for growth in the Loire Valley region of France, and because of grower interest in this region and French acceptance of more acidity in apples, the apple was named for use in France.

Macfree, named and released from the Ottawa program, resembles McIntosh except the fruits are somewhat smaller. The variety has not been tested to any extent in the United States but will probably do well where McIntosh is grown successfully.

Nova Easygro, produced from the Kentville project, is the only variety released with scab resistance from a source other than *Malus floribunda* 821. It has one or more scab resistance genes from the Russian seedling R12740-7A. The fruits resemble Cortland in appearance and taste, but are smaller. This variety also has not been grown to any extent in the United States.

Of the new Co-op series numbers now being patented, two, Co-op 12 and 13 (6), are summer apples with large, highly colored, attractive fruit, firm flesh, and superior keeping qualities. Both have shown storage life up to 6 months. These selections may usher in a new interest in summer

apples as they exhibit a superior flesh type and keeping quality over other summer apples now available to commercial growers.

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### ANALYSIS OF APPLE SCAB EPIDEMICS AND ATTEMPTS AT IMPROVED DISEASE PREDICTION

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Apple scab lends itself to epidemiological research in that infection, particularly during the primary stage, is strongly governed by weather. A frequent student project involves the labeling of leaves as they unfold and then relate disease development on each leaf to the weather that existed when that leaf emerged. With the current emphasis on quantitative and mathematical approaches to epidemiology and pest management, and with major advances in electronics, several research workers throughout the world have undertaken the analysis of apple scab epidemics and the development of faster methods for predicting apple scab infection periods. The intent of this discussion is to review some of these recent efforts including those at Michigan State University.

#### STATISTICAL APPROACHES

Analytis (1) used multiple regression analysis to identify the influence of 16 variables relating to the development of the host on the severity of apple scab infection. Three variables—leaf age (order of unfolding), the date of the first appearance of lesions, and the appearance of the lesions (necrotic or not)— were the only variables of significances. Their order of importance, starting with the most important, was as follows: date of first appearance of lesions, leaf age, and appearance of the lesions. Ordering was accomplished through vector analysis. There was a decrease in susceptibility as leaves became older. Also, late growing shoots were more susceptible than shoots that stopped

growth early.

Analytis(1) also studied the influence of 11 and 15 biological and biometerological variables on the rate of disease increase as measured in successive 5-day periods. Two variables were significant: 1) a variable that described the effect of temperature on spore germination during periods of leaf wetness and 2) the frequency of hourly temperatures in the range of 18-20 C over a 2-day period, 10 days before each disease recording. The first variable was in effect a substitute for Mills infection periods. It accounted for 62.6 per cent of the variability. The second variable was much less important and only accounted for 5.5 per cent of the variability. Other comments on Analytis' paper are found in a chapter on multiple regression analysis by Butt and Royle (2).

#### **COMPUTER SIMULATION**

The development of the EPIDEM simulator for early blight of tomato by Waggoner and Horsfall (9) stimulated the development of other diseases simulators. Thus, EPIVEN was developed by Kranz et al. (5) for the simulation of apple scab epidemics. This simulator was based on EPIDEM except many of the parameters were changed to incorporate data on the development of apple scab. Some parameter estimates were left unchanged until they could be determined experimentally.

Recently, a simulator called VISIM for *Venturia inae-qualis* simulation was developed by Sutton, Bobzin, and Jones. This simulator differed from EPIVEN in several respects. Rather than using an existing disease model, it was developed from biological information on apple scab found in the literature or from laboratory experiments. It was programmed to allow updating through the season. Variables are now being checked in field, laboratory, and growth chamber experiments.

These simulators point up the critical need for research on the relationship of disease potential and inoculum potential, particularly during the primary infection period, to disease severity. Prediction of infection periods is fairly well accomplished by use of the Mills' criteria (6), but predicting the amount of disease that will develop is much more difficult. Knowledge is needed on the susceptibility of apple at different stages, the environmental effects on host susceptibility, the relation of inoculum density to resultant lesion numbers, and the effect of environment on the capacity of the ascospores to infect.

#### ON-LINE FORECAST SYSTEMS FOR APPLE SCAB

The development of a graph (and table) for predicting scab infection from the duration of wetting and the average temperature during wetting by Mills (6), along with the development of spray materials with eradication properties, were significant in improving timing of fungicide application and overall scab control. These developments provided the basis for making apple scab infection predictions and control information available to growers in many

countries through radio, telephone, or postal warnings.

Although infection predictions based primarily on weather conditions are useful, interactions with inoculum levels can lead to erroneous conclusions concerning the pressure on a particular spray program. The Mills' system assumes high levels of inoculum are present, an assumption that is wrong for most commercial orchards. Several warning services included information on potential ascospore discharge by following ascospore maturity and monitoring discharge in freshly collected field samples. However, because of environmental variations, anticipated release determined from laboratory studies may not reflect spore levels in the air during a particular wetting period. We therefore developed a system for utilizing both weather and spore discharge information.

Beginning in spring 1974, an apple scab monitoring program was offered to Michigan apple growers as part of the Michigan Apple Pest Management Project (Fig. 1) (3,4). A hygrothermograph, DeWitt leaf wetness recorder, rain gauge, and two Rotorod samplers with a moisture sensitive control unit were located at each of 12 monitoring sites. Five of the sites were established in conjunction with the Western Michigan Agriculture Weather Service Program (U. S. Department of Commerce, National Oceanic and Atmospheric Administration). Four technicians at three field stations maintained the instruments, collected the environmental data, made the spore counts, and transmitted the data to a computer on the Michigan State University campus in East Lansing.

Environmental data were collected after rainfall or every 3 days during dry periods. If precipitation had occurred, the collectors ("I" rods) on the Rotorod samplers were changed, taken to the field stations, and examined for

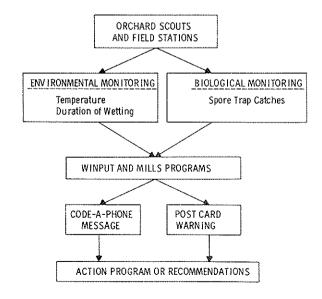


Figure 1.—Block diagram illustrating the basic components and organization of the apple scab monitoring program.

ascospores. If ascospore numbers were high, 10 passes were made at random across the short axis of the collecters, if numbers were low, 20 passes, or roughly one-third of the total sampling area of the collector, were made and the counts adjusted to give a total for each collector. Spore counts and environmental data were entered into a CDC6500 computer at Michigan State University via WIN-PUT and analyzed using MILLS. WINPUT and MILLS are computer programs designed specifically for handling weather information and for predicting scab infection periods. The analysis was made available to fruit growers by the District Extension Horticultural Agents who recorded a 3-minute Code-A-Phone message. Information from the 12 stations was also retrieved at the Michigan State University campus and postcard warnings were sent to all growers in the project.

### AUTOMATED DATA COLLECTION AND PREDICTION

In practice, the monitoring of temperatures and the duration of wetting periods for making Mills predictions is time consuming and most fruit growers usually rely on consultants, chemical sales representatives, or Extension agents for this information. Others resort to applying fungicides on a set schedule.

Timing fungicide sprays for scab over large areas is a problem because of: 1) variations in weather between orchards, and 2) the time required to disseminate the prediction. Increasing the number of weather monitoring sites within an area helps to overcome the first problem but compounds the second problem unless the weather data are available on-line from each site. However, placing the equipment in individual orchards for local predictions would solve both problems.

Automated detection of scab infection periods has been desired for many years. One of the first systems for predicting scab was developed by C. Small in New York. A moisture sensor system activated an alarm clock when rain began. The alarm on the clock was generally set to go off after 9 hours of wetting. The grower, by checking the temperature and current weather information, could estimate if an infection period would occur and if a spray was

Pfleiderer Electronics in Germany, using solid state electronics, have constructed an electronic scab warning system for predicting scab infection periods in the orchard (7,8). The instrument consists of a sensing unit for monitoring the ambient temperature and the duration of wetness. Weather data are stored electronically and the status of an apple scab infection period is provided through a display on the console. The Mills table and information on the effect of relative humidity on spore viability are built electronically into the instrument. Preliminary evaluations of this instrument in Michigan gave favorable results.

A battery-powered, CMOS, microprocessor-based instrument which uses average air temperature, the hours of leaf wetness, and the per cent relative humidity as inputs to a resident predictive model is being developed in Michigan. This instrument differs from the Germany model in that it is programmable, and the criteria for predicting infection periods can be changed as new information becomes available. Moreover, models for other diseases can be added easily to the system.

On the farm monitoring of weather has gained some acceptance. Several growers in Europe are now using the German instrument. In Michigan, some growers own weather monitoring equipment for obtaining the data required to predict apple scab. Some continue to use the instrument developed by Small.

#### CONCLUSIONS

Improvement in the procedures and techniques for predicting apple scab are being attempted in several areas of the world, but most of these approaches have not been evaluated sufficiently to establish their effectiveness under a range of conditions. Prediction of apple scab infection and development beyond that possible with the Mills system, and with sufficient precision for use in pest management programs, is currently hampered by a lack of basic information on the population dynamics of apple scab. Sophisticated techniques for monitoring orchard weather are rapidly being developed and will increase the availability of accurate weather data for use in forecast systems.

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### EVALUATION OF PHYSICAL MODES OF ACTION OF FUNGICIDES AGAINST THE APPLE SCAB FUNGUS

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#### INTRODUCTION

This discussion focuses on the evaluation in the greenhouse of fungicides as formulated products suspendable in water for application to apple trees in the control of scab caused by *Venturia inaequalis* (Cke.) Wint. The procedures described stress individual physical modes of action of fungicides; namely, retention (protection), after-infection, redistribution, presymptom, postsymptom, and systemic. Under controlled laboratory-greenhouse conditions, it is possible to study these actions with a single spray, simulated rainfall, inoculation, temperature, and infection period which would usually be impossible in the orchard. Knowledge of fungicidal modes of action aids considerably in the manipulation of materials and programs in the orchard both in research investigations and in commercial applications.

#### MATERIALS AND METHODS (General)

Certain techniques are basic to all fungicidal mode of action studies including plant culture, spraying, inoculation, environment, and performance assessment.

#### Plant Material

Good vigor and uniform tree growth are the keys to successful and reproducible experiments on scab fungicides. Among the most favorable varieties are Rome Beauty, McIntosh, Macoun, and Niagara.

Nursery-grown apple trees on either natural or size-control (usually EM VII) rootstock are grown in 18-cm clay pots with a substrate composed of one part good field soil, one part sand, and two parts peat moss. The trees are cut back to about 10 cm above the graft union and then placed in the greenhouse. Only one of the developing buds is allowed to grow as a single shoot. Single shooted trees are preferred because they are easily adaptable to precision

spraying and occupy minimal space in the greenhouse and moist chambers. Normal greenhouse maintenance of watering and weekly application of a complete soluble fertilizer with minor elements helps insure growth of good vigor and uniformity. The trees are usable once they develop seven to nine leaves and again each time another seven to eight leaves appear. With proper handling and maintenance, the same trees can be used each year for at least 10 years. At the time a group of trees is used for an experiment, a small piece of cotton string is tied on the petiole of the smallest unfolded leaf (designated "S") on each shoot. As the trees grow, the "S" leaf identifies the test site and facilitates data taking on specific leaves. Usually the "S" leaf and the immediate five older leaves are sprayed and inoculated in fungicide evaluation tests.

#### Foliar Spraying

A basic aim in application of fungicide sprays is the uniform coverage of leaves. The techniques described are for dilute sprays only. Just before spraying, the required amount of material is slurried in water and then more water is added to bring the suspension to the desired volume. Constant agitation of the preparation is necessary for spray uniformity at the time of application. Spray application varies with the needs of specific experiments but basically conforms to one of two types; namely, (1) precision spraying to achieve a specific amount of spray per unit area of upper leaf surface without run-off, and (2) non-precision spraying for complete leaf coverage with runoff of excess. Precision spraying is described under "Fungicide Retention." Non-precision spraying is usually the application with a hand held paint gun or similar sprayer operated under air pressure usually at 25 psi.

#### Inoculation

Apple scab conidia are secured by washing the spores from recently infected apple foliage into a beaker with distilled water delivered from an atomizer at 20 psi. These spores are diluted to a desired volume with distilled water containing 10 ml V-8 juice per liter. In most cases, the concentration is standardized to 70,000 spores/ml confirmed by hemocytometer count. This standardization is necessary for consistency because there is a negative correlation between inoculum level and fungicidal performance (Fig. 2).

The well-agitated spore inoculum is applied at 25 psi by hand held atomizer adapted to an air pressure line. Atomization is continued until the whole leaf is uniformly covered with droplets of inoculum, each approximately 2-3 mm in diameter. Such precise inoculation is necessary for eventual development of well defined, discrete, easily counted scab lesions. Excessive inoculum runoff from leaves may result in uneven lesion development over the leaf. The inoculum is allowed to dry on the leaves to avoid its displacement during subsequent movement of trees to the moist chamber.

#### Infection Period

Infection of inoculated trees is accomplished in chambers with controlled air conditions of temperature and moisture. Apple leaves must be continually wet for spore germination and completion of the scab infection process. This is possible from condensation on the leaves of colloidal water particles from a misting device in the chamber. Temperature is set at the desired level for each test, usually at 20 C which is favorable for infection by the scab fungus. Stratification of mist and temperature level is prevented by a recycling air movement system in the moist chambers. The duration of time that trees are kept in the moist chamber depends on the nature of the experiment.

#### Data Taking

Apple scab infections are first visible on the leaves about 8 days after inoculation, but assessments are not made until 2 or more weeks after inoculation to allow for full symptom expression. Counts are made as actual lesions per leaf on the "S" leaf and each successively older leaf on the shoot to about the fifth or sixth leaf. Notes are also made of types of lesions; i.e., chlorotic or normally sporulating; and of any detectable phytotoxicity from the fungicide.

#### Assessment of Data

In treatment of data from any experiment, it is first necessary to determine from leaf lesion counts which leaves on both sprayed and unsprayed trees became most consistently infected. Then, calculations are made using data from the same aged leaves of every tree in each treatment of the experiment. Usually the composite level of infection, as average number of lesions per leaf, is determined from three or four consecutive leaves of the same age per tree on each of the three trees used for each treatment

For ease of evaluation of fungicidal performance in each

test and development of a final summation from a number of experiments, a Control Rating scale of 0 to 10 was devised from a log10 graph with an arbitrary zero assigned. The zero rating means complete control, and 10, no control. This scale permits the assignment of a numerical control rating for each treatment based on the level of scab infection on the unsprayed trees in each experiment. From these, a final average Control Rating value is readily determined from a number of experiments (Table 1).

The minimal per cent control for ratings 0, 1, 2, etc. is 100, 98, 94, 89, 83, 76, 68, 59, 47, 29, and 0.

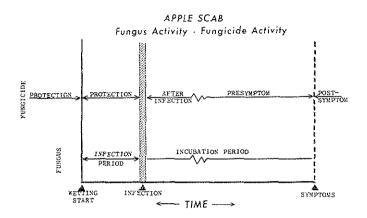


Figure 1.—Type of activity of fungicides applied as sprays to apple trees for control of apple scab (Venturia inaequalis) in relation to the fungus activity during the infection, incubation, and postsymptom periods.

#### MODE OF ACTION TECHNIQUES AND DISCUSSION

Figure 1 shows the relationship between fungus activity and fungicidal mode of action. This, together with a definition given for each mode of action as introduced in this section, will clarify the role of each in the complex scheme of total fungicidal performance.

#### **FUNGICIDE RETENTION**

Fungicide retention is the tenacity of the applied fungicidal residue to plant surfaces under the erosive action of moisture. A fungicide, however potent, is of little practical worth in orchards if it is readily removed from the trees by even light rains leaving the trees unprotected. Good retention is necessary for sustained protection for one or more infection periods.

**Technique.**—Precision spraying of trees is necessary for uniform application of an exact amount of fungicide per unit area of leaf surface in each experiment. The spraying apparatus can be either a commercial type or a researcher designed unit, providing it is controllable, reliable, and gives a consistent spray deposition. The spray nozzle may move over stationary plants or remain in a fixed location with the plants passing beneath on a linear belt or on a turntable.

Table 1.—Disease control rating for fungicides in separate categories of physical mode of action in the control of apple scab (Venturia Inaequalis) in greenhouse evaluations at Geneva, New York.

Fungicide			Fungicide control rating (Apple scab)*					
	Product per 100 gal	Active ppm	Retention 5 cm rain	After- infection 24 hr	Redistri- bution	Pre- symptom	Post- symptom	Systemic
none	~~	-	10	10	10	10	10	10
captan 50 WP	2.0 lb	1200	6	9	5	10	10	10
dodine 65 WP	6.0 oz	293	2	6	ı	1	1	10
zinc-ion maneb 80 WP	1.5 lb	1440	tr	9	1	10	10	10
Glyodin 30% solution	1.0 gt	720	2	9	4		5	10
benomyl 50WP	6.0 oz	225	5	6	-	tr	2	10
fenarimol 12.5 EC	4.3 oz	40	8	2	-	1	9	-
triforine 20 EC	1.5 pt	360	9	4	-	1	5	10
phenylmercuric acetate 10% sol'n	2.0 oz	15	4	tr	-	1	**	10

<sup>\*</sup>Control rating: Minimal % control for ratings 0, 1, 2, etc. is 100, 98, 93, 89, 83, 76, 68, 59, 47, 29, and 0. tr = trace (= greater than 99.5% control).

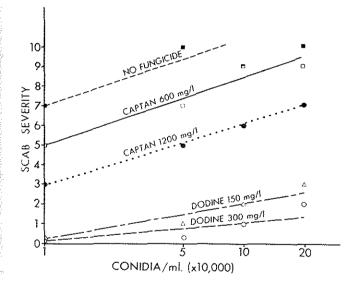


Figure 2.—Severity of apple scab (Venturia inaequalis) on greenhouse trees sprayed with two rates of captan and dodine fungicide, subjected to 5-cm artificial rainfall, and inoculated with different levels of conidia.

Once the spraying system is perfected for precision spraying, reproducible fungicide retention tests are possible each time when employing the same sprayer, pressure, nozzle type, distance between nozzle and plants, alignment of plants with nozzle, and speed of linear movement of either nozzle or plants.

The sprayer which we designed at Geneva delivers the spray under 35 psi air pressure via a solenoid valve for controlled on-off operation through a Monarch #50 nozzle on

an adjustable mount but in fixed position at time of spraying. A thin metal baffle splits the spray pattern, deflecting half as waste and allowing half delivered to the trees. The large orifice nozzle used greatly minimizes nozzle plugging and is thus reliable and efficient. The apple trees are positioned in tandem in one arc (radius 90 cm) on a 1.5 rpm turntable. The Rome Beauty is the preferred apple variety because the young leaves lie flat and expose the full plane surface to the spray. The youngest unfolded leaf ("S") of the three trees of each treatment is positioned at the same distance below the sprayer nozzle by adjusting the height of the potholding platforms on the turntable. This distance between nozzle and "S" leaf is 58 cm. The trees are allowed to pass beneath the spray only once for each application of fungicide. A tared glass slide, positioned horizontally at the same level as the "S" leaf, is also sprayed and weighed to check on precision of spray deposit. This procedure deposits 10 mg of fresh spray per cm2 of leaf surface.

The sprayed trees are allowed to dry thoroughly, usually overnight, before exposure to artificial rainfall as follows: all the trees are positioned in one or two rings at the outer edge of a 2.13-m diameter turntable with all the "S" leaves in the same plant (Fig. 3). The rainfall apparatus consists of a boom of two spraying systems TX26 (#45 core) nozzles 86 cm apart and positioned over the turntable to deliver equal rainfall to all trees (Fig. 4). About 30 cm beneath the nozzles is a horizontally suspended frame 1.3 m by 60 cm with a taut sheet of saran fabric (Lumite No. 5182100) which allows fine drops of spray to pass through to the trees below and also traps water which falls as random droplets from the cloth to the trees. With the nozzles positioned 102 cm above the "S" leaves, there is a delivery of 2.5 cm of simulated rainfall per hour. The actual rainfall is measured

in each test. Although the amount of rain may be varied, 5 cm is used in the usual retention test.

After the trees are allowed to dry for at least 3 hours, they are inoculated with a standardized suspension of apple scab conidia and placed in the moist chamber for infection as described earlier. In routine fungicide retention tests, the trees are left in the moist chamber for 30 to 36 hours and then returned to the greenhouse for normal maintenance and eventual data taking as described earlier. The lesion per leaf fdta is calculated and presented as a control rating which readily identifies the relative retentive property of different fungicides tested (Table 1).

#### AFTER-INFECTION

After-infection activity of a fungicide is its capability of arresting further development of the scab fungus after it had already initiated infection, thereby preventing development of symptoms (Fig. 1). This activity has also been referred to as eradication, curative, and kickback. The term "eradication" may be misleading because it has been used to designate the "burning out" of established scab lesions as with lime sulfur and dinitro compounds. The after-infection properties of fungicides are usually limited within the range of hours to a few days.

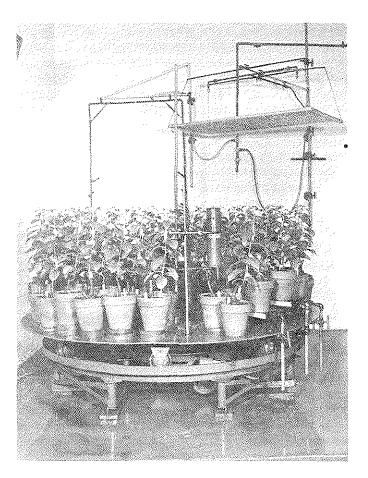


Figure 3.—Single-shooted apple trees on a turntable receiving controlled artificial rainfall in a test to determine retentiveness of fungicides to foliage.

Technique.—Apple leaves are inoculated with a standardized suspension of scab conidia and the trees then held in the moist chamber for infection as described earlier. Initially, fungicides are tested at the 24-hour level to first determine any after-infection activity. In this case, the trees are taken from the moist chamber after an exposure there of 23.5 hours, and the fungicides are applied at 24 hours. Each fungicide is prepared as a dilute spray in water just before application and applied by a paint gun sprayer at 25 psi held about 30 cm from the leaves to achieve a fine spray. With good agitation, the spray is applied to runoff. The trees are then returned to the greenhouse for normal maintenance and data taking as described earlier. Data are collated and presented as a control rating (described earlier) for easy comparison of fungicides for their relative after-infection activity (Table 1). Fungicides with effective after-infection activity at the 24-hour level are further evaluated in tests with spray application made to trees 2, 3, and 4 days after inoculation.

**Discussion.**—It has been convenient for many years to categorize fungicides for their after-infection activity based on effectiveness in hours or days from the beginning of the wetting period because more accurate determination of when the wetting begins is possible than when actual infection occurs under orchard conditions. Actually, during the wet period leading to infection, the fungicide whether existing as a residue or applied during the wet period performs as a protectant in blocking spore germination and/or invasion of the plant (Fig. 1).

#### PRESYMPTOM ACTIVITY

This newly discovered mode of action of fungicides is defined as the disease suppressant activity of a fungicide applied well after infection has occurred but before symptoms appear (Fig. 1). Thus far, presymptom activity for apple scab which has been demonstrated with the fungicides dodine, benomyl, and fenarimol includes the prevention or marked suppression of spore production in lesions. Such lesions are usually chlorotic. The practical value of this activity is the reduction of inoculum and secondary infections.

**Technique.**—The procedures are identical with those described for "after-infection" except that the fungicides are applied beyond their known limits for after-infection activity but prior to the appearance of any symptoms of infection.

About 2 or more weeks after inoculation, two or three leaves of the same age are collected from each of the three trees of each treatment with precautions to avoid cross contamination of samples with spores. In the laboratory, all the spores are harvested from each sample by washing the leaves with distilled water at 25 psi with an atomizer. The spore suspension is brought to a standard volume, agitated thoroughly, and the concentration determined by counts in a hemocytometer. All the scab lesions are counted on the leaf samples. Spore production per lesion is determined from the spore and lesion counts. The relative presymptom

activity of each fungicide can be identified on the basis of spore production per lesions, by degree of spore suppression as expressed either as a percentage compared with unsprayed trees, or by a control rating described earlier and shown in Table 1.

#### POSTSYMPTOM ACTIVITY

Postsymptom activity is the action of a fungicide applied to the plant after appearance of disease symptoms which arrests or inhibits further process of the disease. This may occur in various manners, such as, "burnong out" of scab lesions, restricting further advance of the fungus, or suppressing further sporulation. The procedure described deals only with the latter; namely, the inhibition of production of new scab conidia after spraying.

**Technique.**—The apple leaves are inoculated with scab, the trees are kept in the moist chamber for infection, and then returned to the greenhouse for normal maintenance as described earlier. After 2 weeks after inoculation when the trees have well defined sporulating scab lesions, the spores are washed from about five leaves on each tree with distilled water applied at 30 psi with a paint sprayer. Immediately thereafter, these leaves on three trees per treatment are sprayed with a freshly prepared dilute spray of the test fungicide at desired rate using a paint sprayer at 25 psi. Minimal time should elapse between spore removal and spraying because scab lesions from which spores have been removed can produce thousands of new spores within 2 hours.

About 3 days after spraying, two or three leaves of comparable age from each of the three trees per treatment are harvested for spore and lesion counts. Hereafter, the procedure is the same as for "presymptom" activity.

#### REDISTRIBUTION

Redistribution is the relocation of fungicidal residue on the plant surface. This may be the movement of the particulate fungicide by the physical force of splashing and washing of rains, or by "creepage." The latter is the movement of the fungicide over the surface of the plant part in a film of water extending beyond the actual sprayed area. The "creepage" is readily evident in a glass container of fungicide suspended in water as a film extending up the inner wall of the container a centimeter or more above the original level. Redistribution by solution is the nonparticulate movement by diffusion of a soluble fraction of the fungicide from one site to another in a film of water on the leaf or fruit.

Technique (a) (Potted trees in orchard).—There is no reported greenhouse technique for the evaluation of scab fungicides for redistribution. Limited knowledge on the redistribution of apple scab fungicides is based on a combined greenhouse-orchard procedure. Susceptible apple trees grown in pots in the greenhouse are placed underneath sprayed orchard trees so that they receive dripping water from foliage and not a stream of run-off from

larger branches. The trees are set in the orchard after the orchard sprayed trees are dry, but just before an anticipated rainfall.

After a rainfall of recorded amount and duration, the trees are returned to the greenhouse, inoculated with apple scab conidia, and given an infection period in the moist chamber. The amount of scab infection on the trees indicated the redistributive property of a scab fungicide (Table 1).

Technique (b) (Collected drip water).—Collection units for drip water are simply constructed as approximately 3 feet square wooden frames with legs about a foot long to keep the units off the ground. A sheet of polyethylene plastic is loosely stapled to the frame to form a collection trough. The units are placed underneath sprayed orchard trees as noted above for potted trees. After rain of recorded amount and duration, the drip water from the trees which has collected into each trough is thoroughly agitated to suspend any settled fungicide and a sample taken to the laboratory. The polyethylene sheet of the unit can be replaced for collecting additional samples as desired from subsequent rains.

The orchard sample of drip water is sprayed onto untreated potted apple trees in the greenhouse with a paint gun sprayer. After the trees dry, they are inoculated with apple scab conidia and given an infection period in the moist chamber. The level of infection on the leaves, if any, provides information on the redistributive value of fungicides. Limited use of this technique confirmed the results from the method of using potted trees in the orchard; namely, that the control level from redistribution of dodine is greater than that for captan. In addition to testing on trees, the drip waters can be chemically analyzed for actual fungicidal content.

Discussion.—One or more types of redistribution may prominently figure in the control of apple scab achieved with many fungicides. The same wet conditions which favor scab infection may also favor the redistribution of fungicides which may spread over the entire surface of a susceptible leaf or fruit even though the coverage from spraying was only partial. In addition, the movement of fungicides from leaf to leaf, to fruit, to branches throughout the tree also occurs. A major benefit from fungicide redistribution is the coverage and protection of new growth which had occurred since the previous spray application. Although redistribution usually furthers fungicidal protection, it can also be associated with after-infection, presymptom, and postsymptom activity.

#### SYSTEMIC ACTION

There is a need to clearly define the term "systemic" because it is used freely by many researchers and others with broad and vague interpretations. Many fungicides can be identified as systemic by root absorption from an aqueous or soil substrate and movement within the plant vascular system to aerial parts of the plant where some level of disease control is demonstrable. However, in the

control of apple scab on the apple tree, a woody plant, we must consider the usual manner of fungicide application and confine the definition to *foliar application*. Within this context, the following definition for systemic action would apply: the ingress of the fungicide into a leaf and its movement to one or more leaves (or to fruit) above or below it on the stem at sufficient concentration to exercise meaningful control activity against the disease organism and/or its harmful effects. Effective cumulative levels of the fungicide or its active breakdown products resulting from multiple leaf spraying would fit this definition.

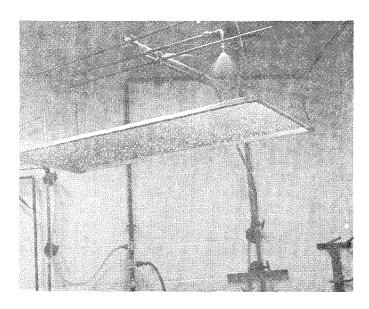


Figure 4.—Rainfall apparatus with horizontal saran screen which provides a rainfall of fine mist and of coarser droplets.

It would be misleading and incorrect to define as systemic action the ingress of a fungicide applied to a part of a leaf and its movement within the leaf restricted to just the one leaf. This type of action has been termed by the author, for want of a better definition, as "local systemic action" or "local intratherapy." Even the term "local systemic action" may be considered a misnomer because "systemic" connotes nonlocal restrictions. Until more suitable terminology is accepted, "local systemic activity" is defined here as follows: ingress of a fungicide into a leaf and its (or modified toxicant) movement within that leaf only at sufficient concentration to exercise in a non-treated area of the leaf some meaningful control activity against a disease organism and/or its harmful effects.

Technique ("General systemic").—Potted apple trees of excellent vigor and growth uniformity in the greenhouse are allowed to develop about 10 to 15 leaves (usually on single-shooted trees). Each fungicide is applied as a dilute spray thoroughly to the top and bottom of all the leaves with a paint gun type sprayer at 25 psi. The trees are maintained normally in the greenhouse until about six new leaves have developed. These leaves are inoculated with a standardized suspension of scab conidia and the trees placed in the moist chamber for infection as described earlier. If more than six new leaves are allowed to develop for inoculation, there is the likelihood that the oldest of the new leaves may develop too low a level of infection to aid in the evaluation of fungicidal performance because of the tendency toward resistance by the mature leaves. After allowing about 2 weeks after inoculation for development of well defined scab lesions, any level of systemic action with the test fungicides would be evident on comparable aged leaves if lesion counts were significantly reduced.

Over the span of 25 years, many experimental and standard fruit fungicides have been tested by this method. To date, none have been found to provide any meaningful level of scab control through general systemic action (Table 1).

#### **GENERAL COMMENTS**

Assessment of the individual physical modes of action of apple scab fungicides exposes the range of differences among these materials. Favorable activity is a decided asset, but unfavorable performance in one or another mode of action need not be too serious if the fungicide has advantages in other modes of action. In the orchard, the total performance of each fungicide accrues from the complementary activities of all modes of action based on the level of the fungicide on the tree for any given infection period. There may be compensating in modes of action in orchard spray programs stemming from successive multiple applications of the same fungicides which may provide overlapping benefits.

All the known and unknown modes of action, together with biochemical modes of action, comprise the total fungicide. These determine the rate of active fungicide needed per tree or per acre, the number and timing of sprays, and the type of orchard program in the control of scab and other diseases. The greater the understanding and intelligent adaptations of these factors, the greater the degree of success associated with the management of orchard disease control programs.

### ANTISPORULANT ACTIVITY OF VARIOUS FUNGICIDES ON APPLE SCAB FOLIAGE LESIONS

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There are many factors which must be taken into account when trying to control apple scab. One factor is the fungicide dosage or rate of application. With the availability of effective protectant fungicides, apple scab can usually be kept under control, especially with high rates of fungicide application. However, with the increased cost of materials and the increased concern over possible deleterious effects on the environment, there is a need to minimize the use of fungicides without reducing the level of disease control.

Another factor which is very important, especially where minimal amounts of fungicides are being considered, is the fungus population both in terms of size and dynamics. Under favorable climatic conditions in the spring, considerable damage may, and indeed often does, occur from primary infections. However, even where the level of primary infection is low, the secondary spores produced from these primary infections can cause infection sufficient to severely damage the apple crop. It therefore becomes important to determine the effects of minimal amounts of fungicides on development of the secondary spores and to determine the capability of these fungicides to reduce considial production and germination.

The purpose of this study was to determine the effects of some of the most widely used commercial fungicides on the available inoculum by measuring their effect on spore production and spore germination. The test fungicides were applied at the commercial rates which are commonly used. The data collected from this study were needed also for the establishment of a computerized simulation model for the prediction of apple scab epidemics.

#### **MATERIALS AND METHODS**

'Staymared' apple trees with visible scab lesions were used in this experiment. The test extended over an 8-week period beginning June 12, 1974 during a period of below normal rainfall. The plots were arranged in randomized complete blocks with six single tree replicates per treatment. The fungicides utilized were Benlate 50W, Cyprex 65W, Polyram 80W, Dikar 80W, and Captan 80W. The experimental treatments were applied as dilute sprays at 550 psi pressure with each tree sprayed to the dripping point. The fungicides were applied at 14-day intervals beginning on June 12. Prior to each application of fungicide, 15 leaves

per tree were tagged, each leaf contained one to three young, active lesions. At intervals of 1, 7, and 13 days after each spray, five tagged leaves were removed from each tree. One lesion from each leaf was removed and the diameter measured, the number of spores per square millimeter of lesion surface determined, and the per cent germination measured. The depression slide technique was employed and counts were made after 24 hours. incubation at 20 C. Statistical analyses were conducted using Duncan's Multiple Range Test.

#### **RESULTS AND DISCUSSION**

Benlate caused a reduction in spore numbers throughout the test period. After one spray of Benlate at 2 ounces plus Manzate 200 at 12 ounces per 100 gallons, a 10 per cent reduction in spore numbers occurred within 1 day after the first spray. This reduction increased to 25 per cent after 13 days. The second spray consisted of Benlate at 2 ounces plus Manzate at 12 ounces plus superior oil at 1 quart per 100 gallons. This treatment resulted in a sustained slow decrease in spore numbers which reached a maximum reduction of 37 per cent 7 days after spraying. The third and fourth sprays contained Benlate at 4 ounces plus Manzate at 12 ounces plus superior oil at 1 quart. This increase in the amount of Benlate caused a significant decrease in spore numbers with a maximum reduction of 55 per cent.

Benlate at 2 ounces reduced spore germination by about 30 per cent 1 day after the first spray and 56 per cent after the 13th day. After the second spray, which contained oil, germination was reduced by 70 per cent after which it remained fairly constant for the remainder of the test period. After the first two sprays, the available inoculum, as calculated by the number of spores times per cent germination, reached a point about 80 per cent below the check and remained approximately at that level for the remainder of the test period.

Cyprex at 6 ounces per 100 gallons caused a small but insignificant decrease in spore numbers by the seventh day after the first spray application. The second spray decreased spore production by 44 per cent for 1 week, but after 13 days, spore numbers were similar to those in the check. Increasing the Cyprex from 6 to 8 ounces resulted in

a 45 per cent decrease in spore numbers and this reduction remained fairly constant throughout the 14-day spray intervals

Cyprex also reduced spore germination. There was a rapid reduction of about 60 per cent in germination after the first spray and maximum reduction of 85 per cent was recorded after 7 days. Following the second spray, germination fluctuated between 8 and 23 per cent. Neither the variation in dosage from 6 to 8 ounces nor the number of days after spraying caused a significant variation in the results following the second spray application. There was a significant difference in available inoculum on all dates, with the lowest levels reached after two sprays at 6 ounces followed by one spray at 8 ounces. After the second spray, the maximum reduction in available inoculum was 91 per cent with an average of 70 per cent for the three successive sprays. Therefore, we concluded that about equal results were obtained using either Benlate or Cyprex at the dosages usually recommended.

Captan had no effect on spore numbers, but it sharply reduced spore germination. At rates of 1.0 and 1.2 pounds active ingredient (equivalent 2.0 and 2.4 lb Captan 50 W), Captan reduced spore germination by about 75 to 85 per cent; this effect remained fairly constant throughout the 14-day interval between sprays. However, at 0.8 pound active ingredient (1.6 lb Captan 50W) spore germination significantly increased between the seventh and thirteenth day, indicating partial failure. Our results generally indicate that Captan at 1.0 pound active ingredient might be expected to reduce the available inoculum by about 65 per cent, but at rates below this level, control may be unsatisfactory.

Polyram at 1.5 pounds per 100 gallons did not significantly reduce spore numbers (16%). The effect on germination was greater (27%), but there was no clear pattern in relation to interval after spraying. Starting with the third spray, Polyram was applied at 2.0 pounds. The data indicated that Polyram at 2.0 pounds reduced the amount of inoculum by about 51 per cent.

There was no significant decrease in spore numbers after application of Dikar at 1.5 pounds per 100 gallons. At 2.0 pounds, applied during the third and fourth sprays, there was consistent decrease approximately 50 per cent after the third spray in spore numbers. Spore germination was reduced by approximately 45 per cent one day after spraying at the rate of 1.5 pounds, but spore germination increased sharply between the second and seventh day. An increase in the amount of fungicide to 2.0 pounds significantly reduced spore germination, but there was still a tendency for partial failure between the second and seventh day after spraying.

Further information was gathered the following season on reducing established apple scab using higher rates of fungicides. The fungicides utilized were Benlate 50W, Cyprex 65W, and Eli Lilly EL 222 EC. Fungicide treatments

were applied as dilute sprays on April 28, May 9 and 23, and June 10. Prior to each spray application, 20 leaves per tree, each with 1-3 sporulating lesions, were selected and tagged. Ten each were removed at intervals of 3 and 10 days after each spray application. The spore counts and germination tests were conducted as previously described.

Cyprex at 12 ounces per 100 gallons of water reduced sporulation by 40 per cent and 69 per cent at 3 and 10 days. respectively, after the first spray. After the second spray, sporulation was reduced by 84 per cent and 89 per cent, respectively. Germination was reduced by 99 per cent and 95 per cent after the first spray and 87 per cent and 96 per cent after the second spray, respectively. Cyprex at 8 ounces per 100 gallons had very little effect on sporulation after the first spray, but after the second spray, sporulation was reduced by 83 per cent. Germination was reduced by 97 per cent 3 days after the first spray but by the tenth day. there had been a 50 per cent recovery. After the second spray, germination had been reduced by 98 per cent and 99 per cent, respectively. The higher rate of Cyprex reduced the available inoculum more than the lower rate after the first spray, but no difference was found after the second spray when all were reduced by 99 per cent.

Benlate at 6 ounces, with or without the oil, affected sporulation and germination about the same. Sporulation was reduced after the first spray by 31 per cent and 79 per cent and after the second spray by 96 per cent and 85 per cent, 3 and 10 days after each spray, respectively. The available inoculum was reduced by approximately 95 per cent after the first spray and 99 per cent after the second spray.

EL 222 at 30 ppm, with or without Benlate at 3 ounces, reduced sporulation by 52 per cent after the first spray and 82 per cent after the second spray. EL 222 plus Benlate showed a greater reduction in germination than EL 222 without Benlate after the first spray with 98 per cent and 92 per cent, and 69 per cent and 61 per cent, respectively. After the second spray, EL 222 with or without the Benlate reduced germination by about 93 per cent to 97 per cent. The available inoculum was reduced slightly better with the Benlate after the first spray, but after the second spray, there was no difference with a reduction of 99 per cent. The Benlate appears to extend the initial effectiveness of EL 222

It is clear from these experiments that either Benlate, Cyprex, or EL 222, if applied at the commonly recommended rates, can be expected to significantly reduce the production and germination of apple scab conidia and thus the scab fungus population on established scab lesions. However, under the conditions of these tests, the fungus population was not reduced to a level which would permit a reduction in the recommended dosage of fungicides. Even with the best fungicide treatment, enough spores remained to initiate serious secondary infections unless adequate levels of fungicide were maintained on the leaf surface.

# SINGLE APPLICATION TREATMENTS FOR APPLE SCAB CONTROL

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The single application technique (SAT) for the control of apple scab (*Venturia inaequalis* [Cke.] Wint.) was suggested from the studies of Yamada and co-workers (22,23,24). They found that applications of certain fungicides to the tops of citrus trees at high dosages provided satisfactory control of melanose (*Diaporthe citri*) and scab (*Elsinoe fawcetti*). The fungicides were applied as sprays or impregnated into nets which were placed over the tops of the trees. Of the fungicides evaluated, Difolatan was the most effective. They attributed the success of this method to the retentive and redistributive properties of the Difolatan.

Chevron Chemical Company introduced the single application treatment for fruit trees in the United States in 1967. Lewis (14) sprayed Difolatan 80W onto apples at a single high dosage of 12.5 lb/100 gal (1.5% by weight) at the 1-cm (½-inch) green stage of flower bud development. He obtained good scab control through the bloom period but not thereafter (Table 1). When the single treatment at 1-cm green was followed by three post-bloom sprays of a mixture of captan and zineb at low dosages, scab control by harvest was almost as good as with 12 full-season sprays of dodine at a conventional rate. No injury to the fruit was noted except when the Difolatan spray was delayed until pre-pink.

Subsequently, the SAT concept for apple scab control has been evaluated by several workers in numerous trials in the United States and Canada. Most of these have been reported in the Fungicide and Nematicide Tests from 1968 to 1976. Only a small portion of the research has been recorded in scientific or trade journals (1,3,10,15,16,17,19).

The 80W formulation of Difolatan was soon replaced with the 4 lb/gal flowable (4F) formulation. A rate of 5 quarts of the latter per 100 gallons (0.6% by weight) of spray on a dilute basis was as effective as a similar rate active ingredient applied as the wettable powder (7). In most seasons, Gilpatrick (2,6) found that follow-up sprays of fungicides at conventional dosages beginning at petal fall were essential for satisfactory scab control until harvest; however, in certain years, he obtained adequate seasonal control with only the SAT without cover sprays (4,5,9).

The optimum timing of the SAT is after the appearance of green flower bud tissue but not after 1-cm green. Sprays applied at later stages have resulted in moderate damage to the cluster leaves and, sometimes, fruit russeting. Dormant sprays in late January were not as effective in con-

trolling scab as those applied at bud burst (1-cm green) (2.4).

The addition of oil to the Difolatan spray at rates normally used for mite and insect control (1-3%) often enhances the effectiveness of scab control. However, the addition of oil tends to increase phytotoxicity, especially to the cluster leaves (8). One report (*unpublished*) of reduction of fruit set following the use of oil with Difolatan has been received from growers when cold weather preceded or followed the treatment. No such damage from the use of Difolatan alone has been reported.

A lower rate of Difolatan 4F, 3 qt/100 gal, provides adequate scab control through the pink stage of apple tree development (9). This reduced single application technique (RSAT) is useful when control of other pests and diseases (against which Difolatan is ineffective) is required at pink and during bloom; e.g., aphids, mites, rust, and powdery mildew. The RSAT is more popular with growers than the SAT because of lower initial costs and high flexibility in spray programming.

Certain other fungicides have shown promise for apple scab control when applied at high dosages as SAT's. Of these, dodine and dithianon have been most promising (2,7,20). However, dodine has been inconsistent and dithianon has not been registered for use on apples in the United States. In general, the dithiocarbamates and benzimidazoles have been ineffective as SAT's.

Gilpatrick et al. (10) studied the possible mode of action of the SAT with Difolatan on apple. They observed that the amount of chemical on wood (twigs) was high immediately after spraying and remained so at least to the end of the bloom, declining sharply thereafter. Difolatan was recovered from unsprayed tissues (new leaves and floral parts) of sprayed trees at levels considered to be adequate for protection against scab from the initiation of bud burst until several days after bloom. Residues, thereafter, declined quickly. Young fruitlets carried relatively high levels of Difolatan even 20 days after bloom, during which time they are most susceptible to scab infections. No residues were detected on the fruit at harvest. These results have been verified by the Chevron Chemical Company and other workers (unpublished).

Szkolnik and Gilpatrick (16,17,19) attributed the success with Difolatan as a SAT to its combined properties of chemical stability, high retentiveness on the apple tree, and good redistributive properties. In addition, Difolatan is toxic

Table 1.—Control of apple scab by a single application of Difolatan (Lewis, 1968).

		% Scab			
Treatment and rate	Number	Leaves:			
per 100 gallons	sprays	Spur	Terminal	Fruit	
Difolatan 80W 12.5 lb <sup>a</sup>	1	0.4	58	34	
Difolatan 80W 12.5 1b <sup>b</sup>	1	2.4	70	32 <sup>đ</sup>	
Difolatan 80W 12.5 lb <sup>a</sup> + Captan 50W 0.75 lb <sup>c</sup> + Zineb 75W 0.75 lb <sup>c</sup>	1 3 3	0.1	10	0.7	
+ 21neb /5W 0.75 1b Cyprex 65W 6.0 oz	12	1.5	6	0.7	
Unsprayed	0	80	96	100	

Spray dates: a = delayed dormant; b = pre=pink; c = June 14, July 13, August 8.

to scab spores at very low threshold levels compared to most other fungicides (18). It has been concluded that Difolatan redistributes by the action of moisture (rains and dews) from sprayed tissues to newly developed unsprayed tissues, thus protecting them from scab infections (10). The importance of the intensity and duration of rains and dews is not clear. Washing rains and drops are considered to play a major role, but other mechanisms of redistribution may be involved; e.g., creepage of water containing Difolatan over the plant surface. In addition, the effect of the SAT on ascospore discharge and maturity or redistribution of Difolatan by vapor action from the sprayed trees or the orchard floor have not been adequately investigated.

The SAT, especially the RSAT, has been accepted by many apple growers as a satisfactory alternative to standard spray practices for apple scab control in the northeastern United States and in eastern Canada. When properly applied and when follow-up sprays are well timed, the technique has usually given adequate scab control. The chief advantage of the SAT is the elimination of three or four sprays during the early part of the growing season, thus reducing the need for critical and constant attention to scab infection periods and timing of sprays. This time and attention can be devoted to other orchard management needs. It has been especially useful in orchards where spraying in the spring of the year is difficult because of wet conditions. The SAT usually does not lead to a reduction in costs for fungicide; but, a real saving in costs of fuel, labor, and equipment is realized.

Growers have applied the Difolatan as a SAT in high volumes of water (100-400 gal/acre; i.e., 1X-4X) with good success. Experience with higher concentrations is limited. Szkolnik and Gilpatrick (21) had variable results when using aircraft (70X) to apply the SAT. However, many growers have obtained satisfactory scab control with the SAT by aircraft. The dosage of Difolatan applied and the thoroughness of coverage are probably of more importance than the volume of spray.

Most use of the SAT has been on full-grown mature trees on standard or semidwarfing rootstocks. Limited trials on smaller trees have been promising, but more research is necessary before SAT's can be recommended on dwarf apple trees.

Hoyt and Gilpatrick (11) have proposed that the SAT be used in apple pest management strategies. A single spray of Difolatan in the spring combined with a postharvest antiperithecial treatment could theoretically reduce in most years, if not eliminate in certain years, the need for conventional scab sprays. The SAT might also be of value in delaying the buildup of tolerance in *V. inaequalis* to selective fungicides by eliminating a high percentage of overwintering resistant inoculum.

A fuller understanding of the SAT mode of action including especially the redistribution mechanism is necessary before this concept will reach its maximum usefulness and acceptability in apple disease management. A computerized prediction model to estimate the probability of adequate protection of scab-susceptible tissues at any time after application would be of great value. Weather, tree growth rate, inoculum potential, and initial Difolatan dosage probably would be key factors to determine the level of residues on critical tissues at any time. Gilpatrick (unpublished) used a bioassay technique to monitor the Difolatan levels on susceptible apple tissue following application of a SAT. Based on residue levels detected, he was able to delay the commencement of conventional sprays by 25 days, and thus eliminated two applications without loss of disease control.

The SAT concepts should also be useful in the control of certain diseases of perennial crops with life cycles similar to *V. inaequalis*. Klos et al. (12,13) found this method to be of value on cherry in the control of leaf spot (*Coccomyces hiemalis*); but, it has not performed well against brown rot (*Monilinia fructicola*).

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### THE STUDY OF TRANSCUTICULAR MOVEMENT OF FUNGICIDES

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#### **ABSTRACT**

Transcuticular movement of fungicides has been studied utilizing isolated cuticles, washing off of fungicide not absorbed, and autoradiography. Each technique of study has inherent deficiencies. A combination of the latter two methods revealed carbendazim (MBC) is absorbed by the cuticle but not readily released within lamina, whereas approximately the same amount of oxamyl is absorbed but this is readily released within the lamina.

The study of transcuticular movement of fungicides using isolated cuticles of apple, coffee, and other plants has been used to compare efficiency of several fungicides by Solel and Edgington (3) and Edgington et al. (1). From this research, it was shown that structural changes such as the butylcarbamoyl group of benomyl produce a marked

enhancement of uptake. Also solubility of the pesticide in the spray solvent is vital. As most fungicides are relatively insoluble in water, most systemic fungicides have been formulated as emulsifiable concentrates to permit a rapid partitioning into the cuticle and thus enhance uptake.

However, the method of isolating cuticles from leaves may bias the results. Hoch's study (2) of cross sections of apple cuticle indicate pectinase causes less alteration than other methods of cuticle removal. While this is important, the results obtained in disease control seem to bear out the conclusions reached by the use of cuticles isolated by either the HCl-zinc chloride or pectinase methods. Another deficiency is the hydrated state of the cuticle in the agar bioassay method. The primary attribute of the isolated

cuticle technique is that it permits the researcher to evaluate passage through the cuticle as a singular process.

A second technique for studying foliar uptake is to wash the fungicide from the leaf surface at varying intervals after treatment. From this the rates of uptake are determined.

A third method is to use autoradiography to visualize uptake and movement from the point of application..

We have recently employed the latter two methods to compare foliar uptake of carbendazim (methyl-2-benzimidazole carbamate or MBC) and oxamyl, a nematicide.

#### **MATERIALS AND METHODS**

Cucumber was chosen for studies of uptake. The plants were grown in the greenhouse to achieve high light intensity and thus develop a cuticle representative of greenhouse conditions. When approximately 1 month old, they were treated with radioactive <sup>14</sup>C-labelled pesticides.

Radioactive oxamyl (oxamyl-1-1<sup>4</sup>C, specific activity 1.23 m Ci/mmole) was supplied by E. I. duPont de Nemours Co., Inc. Labelled MBC (ring-2-1<sup>4</sup>C, specific activity 6.67 m Ci/mmole) was obtained from International Chemical and Nuclear Corp., Irvine, California, U.S.A. Treatment solutions of oxamyl and MBC were made up as follows: oxamyl 2400 ppm containing 600,000 dpm 1<sup>4</sup>C-oxamyl per 100 ul and MBC 380 ppm amended with 0.02 per cent surfactant F containing 600,000 dpm 1<sup>4</sup>C-MBC per 100 ul.

#### Rate of uptake of oxamyl

Fifty microliters were applied per leaf to two mature leaves of four replicate plants. The application was made with a Hamilton microsyringe in the form of small droplets in a band across the base of the leaf, and washings were subsequently taken at 1, 3, 6, and 24 hours and at 1 week. The washings were collected, 2 ml samples were taken and mixed with 15 ml of scintillation fluid (0.03% POPOP, 0.7% PPO, 10% naphthalene in 1,4-dioxane) plus 250 ul of 5 per cent sodium hypochlorite, and the vials were counted in a scintillation counter.

#### Uptake and subsequent translocation

Oxamyl and MBC were used to compare uptake by adaxial and abaxial cuticles. Fifty microliters per leaf were applied to the adaxial and abaxial leaf surfaces with a microsyringe as previously described using four replicates.

After 1 week, the leaf surfaces were washed with 20 ml 95 per cent ethanol to remove pesticide which had not penetrated into the cuticle and the radioactivity determined. After the pesticide had been washed off the leaves, the plants were removed from pots, the roots washed free of soil and after pressing, freeze-dried for 72 hours. Plants were then exposed to Kodak No-Screen X-ray film for one month, and films developed.

#### RESULTS

The results from the oxamyl uptake experiment are listed in Table 1 and plotted in Figure 1. From Table 1 it can be seen that there is an increase in uptake of oxamyl with time, although as the graph in Figure 1 indicates, it is not a linear relationship. The highest correlation was found with a curve of the equation  $y = ax^{b}$  as opposed to a linear equation y = a + bx or an exponential equation  $y = ae^{bx}$ . Further, a linear relationship would result in a y intercept of 58.64 per cent oxamyl absorbed at time 0, which doesn't allow for time for cuticular penetration. The range and standard deviation of the data showed a tendency to increase with time. An  $X^2$  test performed on the data using the equation  $Y = 53.64 \times .067$  determined that the data were significant to  $X^2$ .0005.

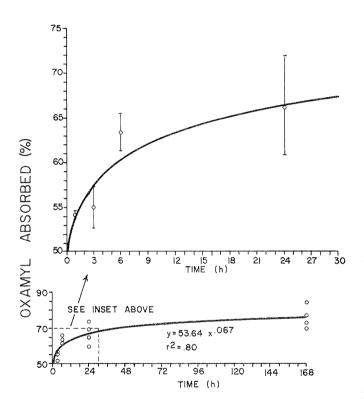


Figure 1.—Percentage oxamyl absorbed by leaf versus time of washing.

The results of the washings from the abaxial and adaxial leaf surfaces are tabulated in Table 2. Although the X² values for each set of four replicates were found to indicate the data were not significant, probably due to the small size of the data sample, the distinction between absorbing surfaces is marked, with more chemical being absorbed through the abaxial leaf surface than the adaxial surface. The two pesticides appear to be taken up about equally.

The autoradiographs of plants (Figure 2) 1 week after treatment showed distinct differences in distribution patterns between oxamyl and MBC. With oxamyl, the spots where the labeled chemical was applied were not visible in

Table 1.—Oxamyl absorbed as determined by wash off from treated leaves.

% Uptake
54.2 ± 0.5 <sup>a</sup>
54.9 ± 2.3
63.4 ± 2.1
66.4 ± 5.5
75.4 ± 6.6

aStandard deviation.

the cuticle. The accumulation was mainly in the leaf margins and veins and sometimes in the buds, with the rest of the plant less distinct. This indicates oxamyl is ambimobile with some downward transport in the phloem.

With MBC, there was a visible difference in cuticular penetration between the upper and lower leaf surfaces where the application spots were clearly defined on the adaxial surface and not so clearly on the abaxial surface. As well, there was an accumulation in the leaf margins and a little in the veins. Most of the MBC was still at the point where the droplets were applied.

#### DISCUSSION

The results from the oxamyl uptake experiment would seem to indicate that greatest uptake occurs over a 6-hour period. A curve reflecting a relationship of this nature between per cent uptake and time would bear out a conclusion regarding penetration similar to the findings of Solel and Edgington (3) on cuticular penetration. That is to say, the per cent uptake is proportional to the amount of chemical on the outside of the cuticle. If 100 per cent of the chemical is initially present and 50 per cent diffuses into the cuticle and 50 per cent of this diffuses into the aqueous chase of the leaf, a concentration gradient will result. However, it will not be static, as the aqueous phase of the leaf is moving under the influence of the pull of the transpiration stream. Consequently, any chemical diffusing the aqueous phase will be swept away and the concentration gradient will have to re-establish itself.

The increase of the standard deviation with time most wely reflects the increasing differences of individual leaves. Studies by Upham and Delp (4) with MBC and benomyl indicate that younger and older leaves vary in their uptake, younger leaves taking up more. This may be due to increased waxes and expansion. Another factor to be considered, although attemps were made to choose leaves of similar size, is increased transpiration pull from leaves with more surface area.

The radioactive counts tabulated for the upper and lower leaf surface applications indicated a trend toward in-

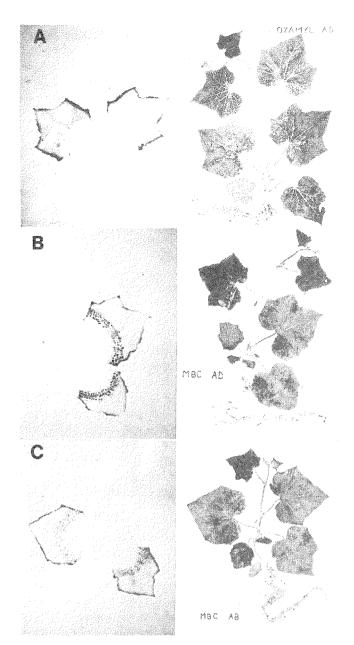


Figure 2.—Autoradiographs and photographs of plants 1 week after treatment with oxamyl (A) and MBC applied to adaxial (B) and abaxial (C) leaf surfaces.

creased absorption by the abaxial leaf surface. Furthermore, MBC and oxamyl appeared to be comparable in uptake through their abaxial surfaces. However, very different patterns of translocation showed up in the autoradiographs, which is to be expected when dealing with an ambimobile chemical on one hand and a pseudoapoplastic chemical on the other hand.

It can be concluded from the MBC remaining in the cuticle and the lack of oxamyl in the cuticle that MBC does not part into the aqueous phase to the same extent as oxamyl. This could be best determined by comparing the partition

Table 2.—Absorption of fungicides by adaxial (Ad) and leaf surfaces.

Chemical applied	Surface treated	Absorbed (% not washed off)
oxamyl	Ab	90.0 ± 4.4ª
	Ađ	75.4 ± 6.6
MBC	Ab	86.0 ± 5.8
	Ađ	75.2 ± 5.4

at standard deviation.

coefficients, "Q" values, for the two chemicals. A lower partition coefficient would be expected for oxamyl, indicating that it would be held less tightly in the cuticle.

Consequently, MBC would tend to be retained more in the waxy layer of the upper cuticle than in the lower cuticle. Oxamyl appears to be lipophilic enough to penetrate through the waxy layer of the cuticle, but hydrophilic enough to readily pass out.

Thus, the results of the leaf wash experiment are very misleading unless accompanied by autoradiographs to detect subsequent movement after penetration into the cuticle.

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# CURRENT AND PROJECTED FUTURE NEEDS FOR NEW FUNGICIDES TO CONTROL APPLE AND PEAR DISEASES

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Plant pathologists worldwide are proud of the success with which fungicides have been used during the past decade. Losses from fungus diseases on apples and pears in many commercial orchards are less than 5 per cent. Considering that there are more than a dozen major fungus pathogens infecting these crops this success with fungicides is truly remarkable. Although diseases in many commercial orchards can be maintained at a low level, the fungicide and application costs necessary to do so have progressively increased annually for several years. The annual per acre cost for disease control may range from \$60 to \$120, depending on diseases present and severity.

Generalized control programs are impractical for an entire region when controlling orchard diseases because of the wide variation in disease occurrence and intensity. This fact and the need to keep pesticide usage to a minimum necessitates the development of specific disease control

programs that were designed to control only the pathogens present in a specific area or orchard. The most prevalent apple diseases in eastern orchards are scab, powdery mildew, rusts, fruit blotches, and rots. Any or all of these may be present during any year, but generally two or three predominate. Some diseases such as scab and powdery mildew are widespread in a number of fruit growing regions while powdery mildew may be the most important one in western orchards. Sooty blotch, fly speck, and fruit rots may be an annual problem in the Cumberland-Shenandoah Valley and only seldomly of commercial concern in New York or Michigan. Wide variation in disease susceptibility among apple and pear cultivars directly affects fungicide performance and should be recognized when devising disease control programs.

Presently used fungicides for apple scab control with few exceptions are basically protective in nature but some are

effective when used a few hours after infection has occurred. Fungicides such as dichlone, captan, maneb, and metiram are of this nature while benomyl and dodine additionally have anti-sporulation properties. Appreciable variation in effectiveness against several important apple diseases exist among this group of fungicides. Dodine is highly effective against scab but is of little value for controlling other major apple and pear diseases. Benomyl has a broad spectrum of activity but does not provide control of apple rust or bitter rot. Fungicides such as captan, thiram, maneb, and metiram have relatively short residual lives against late season diseases such as sooty blotch, fly speck, and fruit rots but provide acceptable control of several other diseases. These late season diseases are generally controlled by using either zineb or folpet in the ast two cover sprays if preceded by applications of other fungicides during the mid-season.

Many factors must be considered when devising control programs for the future. The importance of frequency and rate of application, applicator speed, concentration of the spray mixture, and timing are well recognized. Plant pathologists and orchardists alike are constantly searching for the proper selection and blending of the various factors which will lead to the most practical control program. During the past decade, tolerance of the apple scab fungus as well as other pathogens in some regions to dodine, benomyl, and thiophanate methyl has posed a new problem in the control of these fungi.

Research leading to a more complete understanding of the conditions under which tolerance to fungicides develop orchards is presently needed. Information on the feasibility of using fungicide mixtures or alternating sprays of two or more fungicides to divert the occurrence of tolerance could significantly lengthen usage time of benomyl and related compounds. Tolerance to the benzimidazole compounds of other pathogens such as the powdery mildew fungi on several fruit crops is a distinct possibility in a relatively short period unless preventative measures can be found and instigated soon. It is recognized that the development of tolerance to certain fungicides may be inevitable, but the rate of development is most likely related to the concentration, frequency of application, and duration of usage. Efforts to extend the usage period of highly effective compounds is justifiable in view of the need by growers for such compounds, the high cost of developing new fungicides by industry, and the amount of time and cost dispensed by state and federal workers in the evaluason of new compounds. The ultimate decision on recommending such compounds for grower use under specific conditions should be based on sound scientific data relating to the probability of tolerance developing and the amount of loss likely to be incurred under such an event.

In projecting future fungicide needs, one must consider the types of orchard operations which are likely to exist; types of applicating equipment that may be used; and the type and size of orchard trees which are grown. It is likely that many of the orchards for the next few years will be similar to those presently grown because radical changes in cultural or operational practices are made gradually by most fruit growers. This integration of the old and new as changes are made will of necessity complicate pest management operations.

The tremendous variation in the size of farm, pest management equipment available, and managerial ability are highly significant factors in disease control programs and must be recognized when considering future needs. It is most likely that integrated pest management programs involving both insects and diseases will be used widely in apple and pear orchards in many regions and perhaps worldwide. The management of such programs will require new knowledge, equipment, and chemicals. Some of the knowledge is presently being acquired, and new equipment is being planned or tested. The number of new fungicides in recent years having new chemistry has been encouraging, but emphasis in this area is still needed.

Desirable fungicides for the future should have eradicative or suppressive properties which would allow for their use in different types of management programs. It would be highly desirable to manage diseases such as scab, powdery mildew, or rusts in a manner that would allow establishment at low levels without substantial loss to fruit. This type of fungicide would provide extreme flexibility in disease management programs and permit its use only when the threat of disease development was known to be high. Further, it would be desirable to have fungicides that are more systemic in nature, particularly those that would move downward to the lower trunk and roots to combat fungi infecting this area of the tree. More active systemic movement in the above ground parts would lessen the need for complete and frequent coverage. Most of the present systemics available or being tested have relatively short residual effectiveness. Although this is a highly desirable characteristic in reducing atmospheric contamination, the residual effectiveness period needs to be 2 or more weeks in length to fit the more efficient and less contaminating pesticide programs of the future.

Recommendations on the use of fungicides will need to be relevant to the pathogens present and integrated with known factors helpful in reducing disease levels. There may be many approaches possible, and growers will have to make decisions that best fit their physical and financial situation. For example, the single-application technique (SAT) using captafol may be the most practical approach to apple scab control for a grower with a highly susceptible cultivar and limited applicating equipment or labor during the early spring. This same method, however, is often impractical because of the high cost of the single spray in regions where apple scab is not difficult to control and other diseases such as rusts and powdery mildew are prevalent. New information on the epidemiology of the disease as well as better utilization of present knowledge of fungicide performance will need to be incorporated into future fungicide recommendations. Present knowledge of the effects of temperature, wetting periods, relative humidity, and stage of plant growth on inoculum potential and subsequent disease development should be more fully utilized.

Future disease management programs in summary should be based on as much knowledge as possible on any major factor influencing disease development. Some of the factors that must be considered and carefully incorporated

into the program are: effects of environment on inoculum potential; fungicide type, concentration, application method, and compatibility with other pest management programs; cultivar susceptibility and cultural management scheme; availability, use, and evaluation of new machinery; and availability and proper management and labor.

# DR. W. D. MILLS AND HIS SYSTEM OF PREDICTING APPLE SCAB INFECTION

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I appreciate the invitation to talk for a few minutes about the late Dr. W. D. Mills, both as a human being and as a scientist. His system of predicting apple scab infection on the basis of time and temperature during rain periods is one of the landmarks in plant pathology. As a man, he was a friend, a member of my graduate committee, a critic, and the finest fruit disease diagnostician that I have ever known

Bill was a big man physically, anb most of us referred to him as "Big Bill" to distinguish him from Dr. W. R. Mills who was working on potatoes in New York in the late 1930's. "Big Bill" was in the army for a time and was stationed on the Mexican border and in Europe. I never did learn to separate fact from fiction in some of his tales about that period of his life. I do know that he learned to play poker, to drink alcoholic beverages, and to express himself with one or two well placed pungent adjectives. He fought a continuing battle with his love for alcohol for the remainder of his life, but I saw no evidence that he indulged during working hours. A thoroughly enjoyable day for him was to collect data from 8 a.m. until 5 p.m., buy a couple of drinks, have supper, and then spend the evening with a calculator summarizing the data.

Bill was trilingual, and his conversations with his wife changed from English to Spanish to French as needed to maintain their privacy. He was forthright and positive in his conversation regarding the fruit disease situation in New York. Yet, he tended to pay too much attention to criticism. Through one delay or another, it took him 20 years to reach a point where he was willing to publish relatively precise instructions for timing sprays or dusts during scab infection periods.

Bill had one cardinal rule as an extension fruit pathologist. He wanted one good experiment, preferably one conducted in New York, which he could discuss as a basis for his recommendations for control of each disease. He loved statistics and normally would not use data which had not been analyzed to his satisfaction. He thought that discussion of good data was a highly effective method of teaching.

At the end of World War I, the scab control situation was primitive by today's standards. Lime sulfur had been in use only about 10 years. Little was known about the apple scab fungus and the epidemiology of the disease. In 1919, Keitt and Jones began the work in Wisconsin which led to the publication of their bulletin on scab in 1926. Mills began work in 1924 in New York on the length of rain periods needed for a significant amount of scab infection. As the Wisconsin data became available, he analyzed it with great care and compared the results with those which he obtained in New York. He was particularly interested in the data from Wisconsin giving the number of hours required for infection in moist chamber experiments. Keitt and Jones listed minimum moist chamber exposure periods for leaf infection by ascospores at temperatures varying from 6 C to 26 C, but they did not give a time period for no infection at 15 C. Mills took the Wisconsin data, applied statistical procedures which seemed appropriate, and drew a symmetrical curve which gave the best fit to the moist chamber data. Then, he compared the curve of his own data with the Wisconsin field data and with the reports of a selected group of New York county fruit agents. Some of those county agents did a tremendous amount of work in tagging leaves, recording the length of wet periods, keepmg hygrothermograph records of humidity and temperature, and checking the situation as it developed in the counties. John Van Geluwe, now with Ciba-Geigy, and Cyrus Small, now retired, are two men who were especially helpful.

By the time I arrived on the scene in 1937, Bill had constructed a table which he used in giving precise instructions regarding the time when spraying or dusting with sulfur must be completed during any rain period with temperatures between 42 and 78 F. The basic conclusion was that a significant number of scab infections in an orchard with an abundance of ascospores required about 1.5 times the length of wet period indicated by the Wisconsin moist chamber data. Medium and severe infection required about 2.0 and 3.0 times the wet periods indicated by the moist chamber data. Bill received a weather report by telephone at 3 a.m. every night during the scab season, and a telephone hook-up, whereby each man called about six others, allowed his recommendations to go to every fruit arower in the major fruit districts by about 7 a.m.

By 1937, there had been many improvements in the elemental sulfur fungicides, and many growers were using mem for scab control. Timing the sprays and dusts was a woblem, and part of my assignment was to develop a more effective method of timing dust applications. It soon became obvious that there were several good reasons why the dust should be applied as late as possible before scab infection occurred. Therefore, a major part of the work became a matter of testing dust mixtures before and after danger periods as indicated by Mills' table. After 3 years work on replicated plots and several hundred acres of orchard, I came to the firm conclusion that sulfur dust and the elemental sulfur sprays were effective if applied before time of light infection as indicated by Mills' table. Mills then decided to publish a bulletin on timing sulfur applications for scab control and to include his data on minimum periods required for scab infection. He offered me the junior authorship of the bulletin, but I refused because of concern about his freedom to make recommendations as he saw fit. The bulletin appeared in 1944 with the minimum periods shown in the form of curves which were more conservative and more ambiguous than table which he gave me for use in my work. Data in tabular form were given to John Van Geluwe in 1948 for use in the development of a circular gadget to give to growers. A table was published in the New York fruit bulletin in 1951. So far as I know, Mills made no further changes in the data prior to his retirement.

The use of a system of predicting infection by ascospores led to two major related questions. One concerned the time required for infection by conidia. Mills used the general statement that one-third less time was required for infection by conidia, but the work by Roosje and others has led me to the conclusion that such a general statement is probably incorrect. A second question concerned the need for continuous wetting during scab infection periods. Mills published his conclusion that continuous wetting was not necessary. Roosje has shown that two short wet periods can be separated by 8 to 15 hours without free moisture on the leaves and still result in some infection. The dry period reduces infection without eliminating it until a time period of 8 or more hours has elapsed.

Significant contributions to this subject have, of course, been made by others. In particular, I would recommend the work of Soenen in Belgium and Sproston in Vermont. Papers by Michael Szkolnik have helped a great deal in understanding the activities of the fungus and of fungicides during infection periods.

In closing, I want to say something about the relationship of the work of Mills to that of Dr. James Hamilton and of Dr. Michael Szkolnik during Mike's earlier years. I see no conflict of any significance in the work. Mills and Hamilton were contemporaries, and they competed with each other. Both men would have been quite happy to control the fruit disease work in New York. Hamilton's work, and later that of Hamilton and Szkolnik, helped to provide the fruit grower with better fungicides, extended the period from the beginning of rain until fungicide application must be completed, and, through the introduction of better fungicides, eliminated most of the partial failures which had occurred with sulfur in spite of reasonably good orchard management. There are some instances in the literature, even today, where apparent conflicts are created by statements which are made in an effort to explain a complex situation to growers, especially concerning after-infection control by fungicides. However, these conflicts are not important compared to the tremendous advances in scab control made by the workers at Cornell and Geneva.



