This is to certify that the

thesis entitled

MODE OF ACTION, POSTINFECTION CONTROL CHARACTERISTICS AND SYSTEMIC PROPERTIES OF SELECTED TRIAZOLE AND IMIDAZOLE FUNGICIDES FOR USE AGAINST VENTURIA INQEUQALIS

presented by

Randall David Kelley

has been accepted towards fulfillment of the requirements for

Master of Science degree in Botany & Plant Pathology

Alan L. Jones

Major professor

Date October 20 1980

MODE OF ACTION, POSTINFECTION CONTROL CHARACTERISTICS AND SYSTEMIC PROPERTIES OF SELECTED TRIAZOLE AND IMIDAZOLE FUNGICIDES FOR USE AGAINST *VENTURIA INAEQUALIS*

By

Randall David Kelley

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1980

ABSTRACT

MODE OF ACTION, POSTINFECTION CONTROL CHARACTERISTICS AND SYSTEMIC PROPERTIES OF SELECTED TRIAZOLE AND IMIDAZOLE FUNGICIDES FOR USE AGAINST *VENTURIA INAEQUALIS*

By

Randall David Kelley

Three fungicides (CGA-64251, bitertanol, and phenapronil), for use against apple scab, were evaluated for postinfection control and effect on lesion development. In greenhouse studies, fungicides gave complete control when applied up to 3 days after inoculation, although chlorotic lesions were noted. In field studies, CGA-64251 and bitertanol gave control if applied within 3 days after an infection period or if two sprays were applied 1 wk apart, starting either 2 days before sporulation was predicted or 2 days after lesions were observed. Scanning electron microscope examination of lesions showed fungicides prevented conidia from maturing. A positive correlation existed between delay time and percent mature conidia. Attempts to germinate conidia from sprayed lesions were unsuccessful. SEM examination of chlorotic lesions revealed subcuticular hyphae and deformed surface growth. Isolations from washed chlorotic lesions were successful. X-ray autoradiographs and scintillation counts showed both upward and downward movement, with bitertanol least systemic. Equilibrium of transcuticular movement required from 12 hr to 3 days.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

APPENDIX B

APPENDIX C

GENERAL INTRODUCTION AND LITERATURE REVIEW

Control of apple scab, caused by Venturia inaequalis (Cke.) Wint., has, in recent years, relied heavily on two fungicides; benomyl and dodine. Numerous reports of resistance to benomyl, involving many organisms (10), including V. inaequalis, indicate that its future as an effective fungicide is limited, and many orchards already have levels of resistance which make its use impossible. Dodine resistant strains of the apple scab fungus exist throughout the northeastern United States (11, 14, 18). The loss of these chemicals is particularly distressing because benomyl, and to a lesser extent dodine, could be applied effectively after infection had occured, thus facilitating their use in pest management schemes.

During the past 12 yr, several fungicides which inhibit ergosterol biosynthesis and membrane function have been tested for apple scab control. The pyrimidine fungicides triarimol, fenarimol and nuarimol and the piperazine fungicide triforine have been studied most extensively, and were found to control apple scab when applied after the onset of infection (6, 8, 13, 16, 17). Recently, certain triazole and imidazole fungicides, possessing protective and curative activities against many Ascomyceteous fungi, and which inhibit ergosterol biosynthesis in a manner similar to the pyrimidine fungicides, have become available for testing (1, 2, 20).

Two triazole fungicides: CGA-64251 (1-((2-(2,4-Dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole) and bitertanol, and the imidazole fungicide phenapronil were chosen for study, and an integrated set of experiments was initiated to assess the extent of their postinfection control abilities, and to examine their effects on apple scab lesion and conidial development and morphology. In addition, experiments were undertaken to determine characteristics of movement and localization within the plant; as the ability of a fungicide to enter the plant and the extent and speed of its movement after entry determine, to large extent, the optimal application conditions and the level and type of control to be expected. Solel and Edgington and Edgington et al. (5, 19) have studied transcuticular movement of fungicides on apple leaves, and Interieri (9) and Fuchs and Ost (6) have reported the use of x-ray film autoradiographs for visualization of distribution and uptake.

Nusbaum (12), Preese (15), Corlett et al. (3), and Hammill (7) have produced excellent studies of germination, infection, and lesion development of the normal untreated fungus, and a more recent report by Corlett et al. (4) dealt with the morphological effects of benomyl on V. inaequalis. Their description of preparation of specimens and the photographs supplied with the article suggest that the collapse of surface structures which they noted is more likely an artifact of preparation than a direct effect of the fungicide. However, 75-90% of annellides in treated lesions had ceased development at the primary conidium stage with rounded

immature spores, similar to structures noted in the present study. A comparable morphological study on fungicide treated Penicillium of citrus was reported by VanGestel (22).

Szkolnik (21) recently discussed the methods and value of various approaches to fundamental fungicide research. His analysis of the various possible relationships between spray timing and the stages of disease development was used as the logical basis for portions of the present study.

The data from this integrated set of experiments make it possible to determine the way in which these new fungicides might be most effectively incorporated into pest management programs.

LITERATURE CITED

1. Buchel, K. H., W. Kramer, W. Meiser, W. Brandes, P. E. Frahberger, and H. Kaspers. 1979. Chemistry and properties of phenyl-triazole-methanes, a highly active class of new azole fungicides. Abstract 475 in Abstracts of Papers, IXth Int. Congr. of Plant Protection, Washington, D.C.

2. Carley, H. E. 1979. A new multiple use fungicide: α-butyl-α-phenyl-1H-imidazole-1-propanenitrile. Phytopathology 69:1023.

3. Corlett, Michael, James Chong, and E. G. Kokko. 1976. The ultrastructure of the Spilocaea state of Venturia inaequalis in vivo. Can. J. Microbiol. 22:1144-1152.

4. Corlett, Michael, and R. G. Ross. 1979. Morphology of Spilocaea pomi on untreated and benomyl-treated McIntosh apple leaves. Can. J. of Pl. Path. 1:79-84.

5. Edgington, L. V., P. P. Q. deWildt, K. Jacques, and J. Psutka. 1976. The study of transcuticular movement of fungicides, in Proceedings: Apple and pear scab workshop, pp. 32-35, A. L. Jones and J. D. Gilpatrick, eds., N.Y. Agric. Exp. Stn., Special Report 28, 38 pp.

6. Fuchs, A., and W. Ost. 1976. Translocation, distribution and metabolism of triforine in plants. Archives of Environmental Contamination and Toxicology 4:30-43.

7. Hammill, T. M. 1973. Fine structure of annellophores IV. Spilocaea pomi. Trans. Br. Mycol. Soc. 60:65-68.

8. Hoch, H. C., and M. Szkolnik. 1979. Viability of Venturia inaequalis in chlorotic flecks resulting from fungicide application to infected Malus leaves. Phytopathology 69:456-462.

9. Interieri, Cesare, and Kay Ryugo. 1972. Enhanced penetration and translocation of a fungicide following foliar treatment of seedlings with growth retardants. Plant Dis. Rep. 56:590-592.

10. Jones, A. L., and G. R. Ehret. 1976. Tolerance of fungicides in Venturia and Monilinia of tree fruits, in Symposium: Resistance of Plant Pathogens to Chemicals. The American Phytopathological Society, Inc., St. Paul. MN. 52 pp.

11. Kappas, A., and S. G. Georgopoulos. 1970. Genetic analysis of dodine resistance in Nectria haematococca (syn. Hypomyces solani). Genetics 66:617-622.

12. Nusbaum, Charles J., and G. W. Keitt. 1938. The cytological study of host-parisite relations of Venturia inaequalis on apple leaves. J. Agri. Res. 56:596-618.

13. Pearson, R. C., M. Szkolnik, and F. W. Meyer. 1978. Suppression of cedar apple rust pycnia on apple leaves following postinfection applications of fenarimol and triforine. Phytopathology 68:1805-1809.

14. Polach, F. J. 1963. Genetic control of dodine tolerance in Venturia inaequalis. Phytopathology 63:1189-1190.

15. Preese, T. F. 1963. Micro-exploration and mapping of apple scab infections. Trans. Br. Mycol. Soc. 46:523-529.

16. Prusky, D., Benjamin Jacoby, and A. Dinoor. 1979. Is the accumulation of a systemic fungicide at the infection site related to its eradicative action? Pestic. Biochem. Physiol. 12-75-78.

17. Ragsdale, Nancy N. 1975. Specific effects of triarimol on sterol biosynthesis in Ustilago maydis. Biochemica et Biophysica Acta 380:81-96.

18. Ross, R. G., and R. J. Newbery. 1977. Tolerance of Venturia inaequalis to dodine in Nova Scotia. Can. Plant Dis. Surv. 57:57-60.

19. Solel, Z., and L. V. Edgington. 1973. Transcuticular movement of fungicides. Phytopathology 63:505-510.

20. Staub, T., F. Schwinn, and P. Urech. 1979. CGA-64251, a new broad-spectrum fungicide. Phytopathology 69:1046.

21. Szkolnik, Michael. 1978. Techniques involved in greenhouse evaluation of deciduous tree fruit fungicides. Ann. Rev. Phytopathol. 16-103-129.

22. Van Gestel, J., and J. Van Reempts. 1979. Scanning electron microscopy of Penicillium italicum on oranges treated with R-23979, an imidazole derivative. Plant Dis. Rep. 63: 283-287.

PART I

POSTINFECTION CONTROL OF APPLE SCAB WITH CGA-64251, BITERTANOL, AND PHENAPRONIL

INTRODUCTION

In the development of pest management programs for apple scab, caused by Venturia inaequalis (Cke.) Wint., highly effective fungicides are needed to control disease after the identification of apple scab infection periods. Currently, the availability of fungicides for after-infection control is limited because of fungicide resistance, because some fungicides can only be used early in the growing season to avoid phytotoxicity problems, and because public agencies have cancelled or delayed registrations. During the past 12 yr, several fungicides which inhibit ergosterol biosynthesis and membrane function have been tested for apple scab control. The pyrimidine fungicides triarimol, fenarimol and nuarimol and the piperazine fungicide triforine have been studied most extensively, and were found to control apple scab after the onset of infection (6, 8, 12, 14, 15). Recently, certain triazole and imidazole fungicides, possessing protective and curative activities against many Ascomycetous fungi, and which inhibit ergosterol biosynthesis in a manner similar to the pyrimidine fungicides, have become available for testing (4, 5, 16).

Two triazole fungicides: CGA-64251 (1-((2-(2,4-Dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole) and bitertanol, and the imidazole fungicide phenapronil were studied.

The objectives were to determine the extent of their postinfection control abilities, to examine their effects on apple scab lesion and conidial development and morphology, and to establish how these new fungicides might be incorporated into apple scab control programs.

MATERIALS AND METHODS

Laboratory Studies

Monoconidial isolations of *V. inaequalis* were made by rubbing detached lesions across the surface of potato dextrose agar (PDA) and transferring single germinating conidia to fresh PDA in petri plates. Conidia, produced on cheesecloth wicks saturated with 4% malt extract broth (19), were removed by adding sterile water to drained culture bottles and shaking vigorously. Sterile Millipore filter discs (13 mm diameter) were placed on PDA and 0.3 ml of a fungicide suspension was applied to each disc. Sterile water was applied to control discs. A spore suspension (0.3 ml) was applied to each disc and germination of the conidia was observed 3, 6, and 21 days later.

Greenhouse Studies

Actively growing single shooted McIntosh apple (*Malus pumila* Mill.) trees in pots were inoculated with a suspension of conidia of *V. inaequalis* (3×10^5/ml). The suspension was made by washing infected apple leaves with distilled water, and was atomized onto the trees 1 hr before placing them in a mist chamber at 20 C for 47 hr. The youngest leaf on each shoot was tagged for later reference.

After inoculation, trees were placed on a cheesecloth enclosed bench in the greenhouse. The cheesecloth was wetted daily to maintain relative humidities of 80 to 100%. At different intervals after inoculation, four plants for each fungicide treatment were removed from the enclosure, sprayed with fungicide and, after the deposit dried, returned to the enclosure. Eight inoculated plants were left unsprayed as controls.

Data were recorded 18 days after inoculation by visually estimating (i) the leaf area covered with sporulating lesions and (ii) the leaf area covered with both normal and non-sporulating (chlorotic) lesions, using a standard diagram for comparison (18). The four leaves below the tagged leaf were evaluated. Fungal development within the chlorotic lesions was determined by removing with ethanol, chlorophyll from several leaves with chlorosis and staining the fungus with basic fuchsin (13). The experiment was done twice.

Field Studies

In 1979 an orchard of 3-yr-old McIntosh apple trees on M26 rootstock was sprayed to runoff with a handgun at 500 psi. Treatments were arranged in a randomized complete block design with four blocks and three trees per replicate. Data were taken at about 2-wk-intervals from mid-June to mid-September on 20 terminals per replicate.

In 1980 an orchard of 5-yr-old McIntosh apple trees on M7 rootstock was used. Treatments were replicated four times in a

completely randomized design using single tree plots. Tags were tied to the youngest expanded leaf after critical infection periods to identify susceptible leaves exposed to infection. Data were taken on 30 fruit spurs and 20 terminals per replicate.

Infection periods were predicted with a microprocessor-based instrument placed between two apple trees and about 1.5 m above ground level. This instrument, a modification of a unit described by Jones et al. (9), monitored temperature, rainfall, leaf wetness, and relative humidity in the orchard. Incubation periods were estimated based on the average temperature during the infection periods by using the Mills table (11).

Possible effects of the fungicides on conidial morphology and development were determined by examining lesions with an ISI Super III scanning electron microscope (International Scientific Instrument Corp., Santa Clara, CA 95050). Leaf disks with individual lesions were cut from randomly collected leaves with a cork borer, fixed in phosphate buffered 4% gluteraldehyde for 24 hr. washed twice in 0.1 M phosphate buffer (pH 7.3), and post-fixed in a 1:1 mixture of 0.2 M phosphate buffer and 2% OsO_4 for 24 hr. Samples were washed again in phosphate buffer, dehydrated in an ethanol series and critical point dried. Dried specimens were mounted on stubs and coated wtih a 20 angstroms thickness of gold in a sputter coater. In addition, lesions were rubbed across the surface of PDA to remove the conidia and germination was evaluated after 24 and 48 hr.

To determine the viability of the fungus within chlorotic lesions, isolations were attempted using the procedures of Hoch and Szkolnik (8). Leaves were collected on 3 June from each replicate of treatments showing chlorotic lesions, and washed for 5 hr in running tap water. Twenty chlorotic lesions per treatment were removed with a cork borer, dipped briefly in 70% ethanol and cut into quarters. Each set of quarters was placed in a petri dish containing 3% malt extract agar amended with 250 ppm streptomycin. Plates were examined for growth of V. inaequalis after 20 days at 20 C. Unsprayed controls were included for comparison.

RESULTS

Laboratory Studies

Phenapronil (1235.8 µg active ingredient (a.i.)/ml), bitertanol (599.2 µg a.i./ml) and CGA-64251 (37.4 µg a.i./ml) were applied to Millipore filters. Conidia on all filters germinated, but germ tubes of fungicide-treated conidia were short, distorted, swollen, and branched, often with a dark swollen structure at each hyphal tip (Figure 1).

Greenhouse Studies

Phenapronil (617 µg active ingredient (a.i.)/ml), bitertanol (149.8 and 299.6 µg a.i./ml) and CGA-64251 (18.7 µg a.i./ml) were applied to trees 2, 3, 4, 4.5, 5, and 5.5 days after inoculation. Two fungicides: (i) fenarimol (EL-222 12.5% EC) at 42 µg a.i./ml from Elanco Products Co., Indianapolis, IN 46206 and (ii) phenyl mercuri triethanol ammonium lactate (Puritized Agricultural Spray (PAS) 7.5% liquid) at 93.8 µg a.i./ml from Niagara Chemical Division of FMC Corp., Middleport, NY 14105, which have exhibited good eradicative properties in the past, were included for comparison.

In several treatments, particularly those where fungicide was applied 4 days or more after inoculation, both normal sporulating lesions and chlorotic flecks or yellow nonexpanding and non-sporulating lesions were present (Table 1). CGA-64251, bitertanol

Figure 1.--Germinated conidium of Venturia inaequalis on filter paper saturated with 12.5 µg of CGA-64251. Arrows indicate swollen areas at hyphal tips.

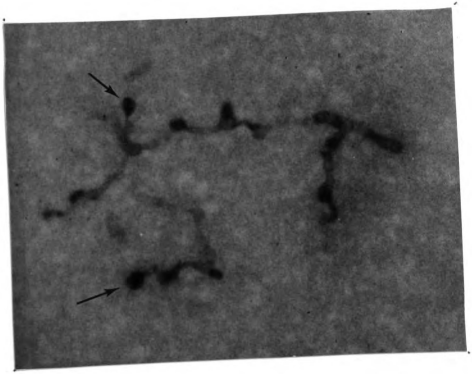


TABLE 1.--After-infection control of scab on potted apple trees in the greenhouse by fungicides applied at various times after inoculation.

Treatment[y]	Dosage (µg a.i./ml)	Leaf Area Infected 18 Days After Inoculation											
		2 days[v]		3 days		4 days		4.5 days		5 days		5.5 days	
		Total[w] (%)	Normal[x] (%)	Total (%)	Normal (%)	Total (%)	Normal (%)	Total (%)	Normal (%)	Total (%)	Normal (%)	Total (%)	Normal (%)
Fenarimol 12.5% EC	41.9	0	0	4	0	23	0	5	0	8	0	24	5
PAS (mercury)[z]	93.8	1	0	8	2	26	8	36	7	34	9	15	2
CGA-64251 10% W	18.7	1	0	0	0	10	0	8	1	18	4	22	7
Bitertanol 50% W	149.8	14	2	27	3	26	12	35	10	27	0	31	3
Bitertanol 50% W	299.6	11	0	6	0	42	0	48	5	42	1	33	5
Phenapronil 2#EC	617.9	0	0	2	0	8	0	11	0	15	3	35	11

[v]Days refer to the interval between inoculation and application of fungicide.

[w]Percent leaf area covered with normal sporulating lesions and with chlorotic flecks.

[x]Percent leaf area covered with normal sporulating lesions.

[y]Unsprayed controls (average of 8 plants) showed 49 and 37% of the leaf area covered with total and normal lesion types, respectively, 18 days after inculation.

[z]Puritized Agricultural Spray (PAS) containing 7.5% phenyl mercuri triethanol ammonium lactate.

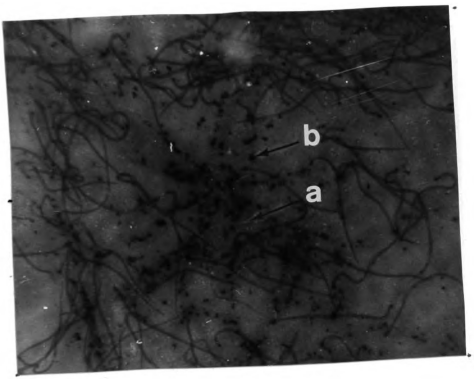
at 299.6 µg a.i./ml, and phenapronil were similar in effectiveness to fenarimol and PAS when applied 2 and 3 days after inoculation. At 4 days, the percent leaf area covered wtih chlorotic flecks was high for all fungicides except phenapronil and CGA-64251, but only leaves sprayed with PAS and bitertanol at 149.8 µg a.i./ml exhibited normal lesions. At 4.5, 5, and 5.5 days, all treatments continued to suppress the development of normal lesions to some extent compared to unsprayed trees.

Leaves with chlorotic flecks retained the basic fuchsin stain in the chlorotic areas of the leaves, indicating the lesions contained fungal growth (Figure 2).

Field Studies, 1979

CGA-64251 10% W (18.7 µg a.i./ml) was evaluated in five postinfection spray schedules as follows: (i) an after-infection eradication program with treatment applied within 3 days from the beginning of wetting periods predicted to give infection; (ii) two presymptom eradicant programs, the first with treatments applied 2 days before lesions were predicted to appear, and a second program where the initial spray was repeated 1 wk later; and (iii) two postsymptom eradicant programs, the first with treatment applied 2 days after lesions were visible and a second program where the initial spray was repeated 1 wk later. A protective spray schedule was included for comparison. Timing of the sprays in relation to predicted infection periods is shown in Figure 3.

Figure 2.--Apple leaf, with chlorophyll removed, stained with basic fuchsia to detect subcuticular hyphae (A) and occasional surface growth (B) of Venturia inaequalis in chlorotic lesions.


b
a

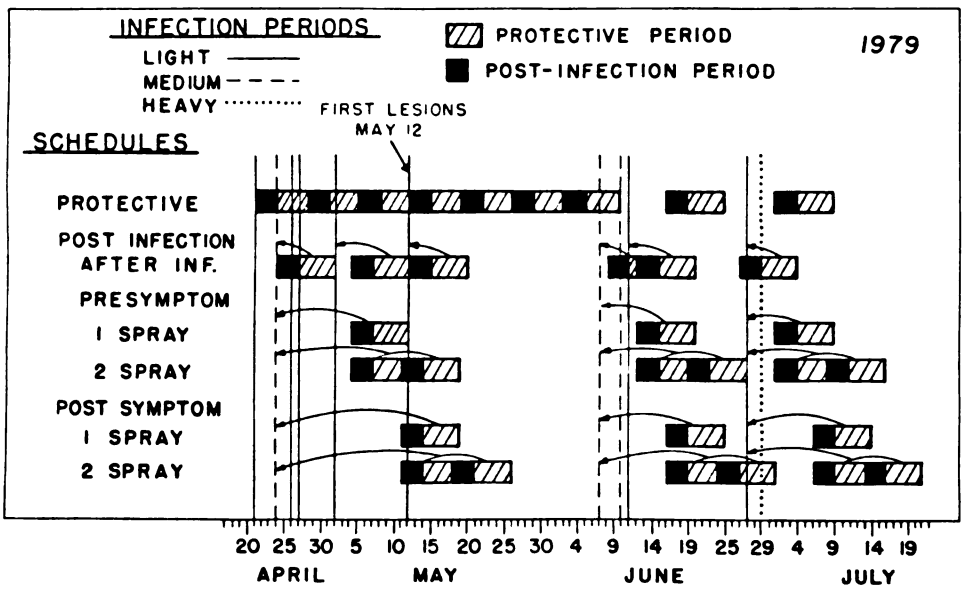


Figure 3.--Schedule of sprays applied in 1979 in relation to predicted infection periods. The date for each spray corresponds to the right margin of the black squares. For purposes of interpreting the possible effect of each spray, a 3-day postinfection period and a 5-day protective period are illustrated. The 3-day postinfection period is from greenhouse studies (Table 1), but the 5-day period for protection is an estimated time. Arrows designate initial infection period for which spray or spray sequence was applied.

The pre- and postsymptom eradicant programs with a repeat spray 1 wk after the first, resulted in significantly more control than the one-spray eradicant programs, and were not significantly different from the protective schedule in effectiveness, even though three fewer sprays were applied (Table 2). The presymptom sprays on 7 and 14 May were applied for infection periods beginning 24, 26, and 27 April and 2 and 12 May; sprays on 15 and 22 June were for infection periods beginning 7, 10, and 11 June; and sprays on 4 and 11 July were for infection periods on 27 and 29 June. The postsymptom eradication sprays for these infection periods were applied 14 and 21 May, 19 and 26 June, and 9 and 16 July. No susceptible tissue was present during the light infection period starting 21 April.

The after-infection schedule was not significantly different in effectiveness from the protective schedule on 11 July, but significantly more scab was recorded on 1 and 27 August (Table 2). However, the last after-infection spray was applied on 29 June, 2 to 3 wk earlier than the last pre- and postsymptom sprays.

Field Studies, 1980

Bitertanol 50% W (299.6 µg a.i./ml) and CGA-64251 10% W (18.7 µg a.i./ml) were evaluated in three postinfection spray schedules as follows: (i) an after-infection eradication program with treatment applied within 3 days from the start of wetting periods predicted to give infection; (ii) a presymptom eradicant program with one spray applied 2 days before lesions were predicted

TABLE 2.--Control of scab on McIntosh apple with protective and postinfection eradicant treatments of CGA-64251 10% W applied in dilute sprays at 18.7 µg a.i./ml under orchard conditions in 1979.

Treatments	Number of Sprays	Incidence of Scab on Terminals[y]						
		6 June		11 July		1 August		27 August
		Leaves Infected (%)	Lesions per Terminal[z]	Leaves Infected (%)	Lesions per Terminal[z]	Leaves Infected (%)	Lesions per Terminal[z]	Leaves Infected (%)
Protective Treatment:								
7-day-schedule	9	0	0	0 a	0 a	3 a	2 a	16 a
Postinfection Treatments:								
After-infection schedule	6	0	0	3 a	1 a	17 b	20 a	42 b
Presymptom schedules								
One spray sequence	3	17	3	41 c	39 c	53 d	115 c	70 cd
Two spray sequence	6	1	0	5 a	2 a	8 ab	6 a	16 a
Postsymptom schedules								
One spray sequence	3	5	1	21 b	16 b	36 c	52 b	55 bc
Two spray sequence	6	3	1	2 a	1 a	6 ab	4 a	15 a
Untreated (controls)	-	24	7	55 c	128 c	79 e	---	84 d

[y]Means followed by the same letter did not differ significantly (P = 0.05) using Duncan's Multiple Range test on arcsin-transformed percentage data and log transformed lesion data.

[z]The mean number of leaves per terminal was 7.5, 22.0, 23.9 and 26.3 on 6 June, 11 July, 1 August, and 27 August, respectively. There was no significant difference between mean number of leaves on different treatments for any of the dates.

to appear and a second spray 1 wk later; and (iii) a postsymptom eradicant program with a spray applied 2 days after lesions were visible and a second spray 1 wk later. Two protective spray schedules, one at 7-day and the other at 14-day intervals, were included for comparison. Timing of the sprays in relation to predicted infection periods is shown in Figure 4.

Data taken on 22 May indicated the control of scab from infection periods beginning 27 and 28 April. After-infection schedules with both compounds were as effective as protective schedules in controlling scab from these infection periods. Pre- and postsymptom schedules, regardless of chemical, were not significantly different from each other or from the untreated trees. Since the first scab lesions were observed on 13 May, the presymptom spray of 12 May was applied too late to be considered truly presymptom. Data taken on 5 June indicated a slight increase in scab on leaves of fruit spurs, but no major changes in the effectiveness of the treatments.

The severity of the 13 and 17 May infection periods is illustrated by the data taken from terminal shoots on 6 June, since the buds that gave rise to these shoots were dormant during the infection periods in April. Data for this date indicate that bitertanol gave better control when applied as a protectant or if applied soon after infection, while CGA-64251 gave better control when applied later in the incubation period. Chlorotic nonsporulating lesions were also noted at this time, and examination of tagged leaves indicated these lesions were from the heavy infection

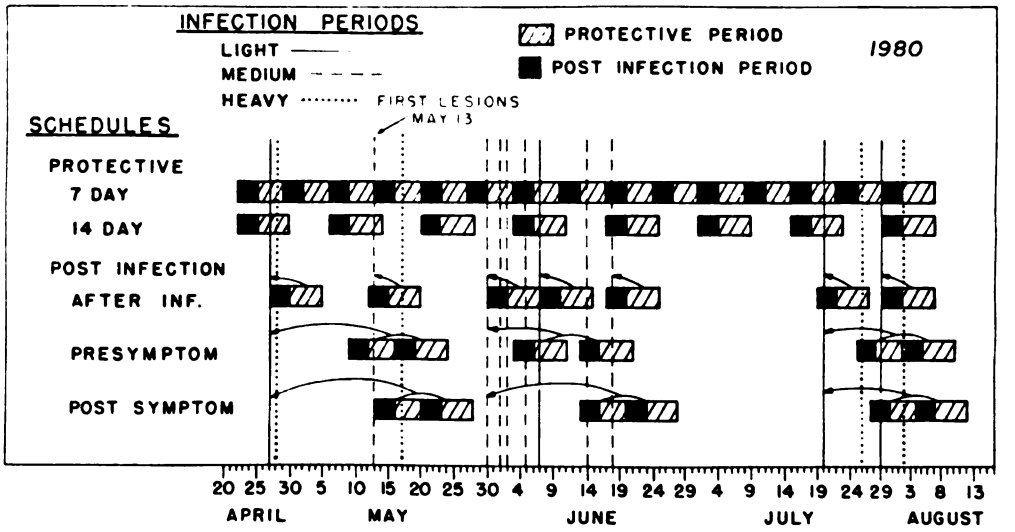


Figure 4.--Schedule of sprays applied in 1980 in relation to predicted infection periods. The date for each spray corresponds to the right margin of the black squares. For purposes of interpreting the possible effect of each spray, a 3-day postinfection period and a 5-day protective period are illustrated. The 3-day post-infection period is from greenhouse studies (Table 1), but the 5-day period for protection is an estimated time. Arrows designate original infection period for which spray or spray sequence was applied.

period of 17 May when 3.02 cm rain, 30 hr wetting, and a mean temperature of 13.3 C were recorded. Similarly, chlorotic lesions were noted on 24 June following a series of five infection periods between 30 May and 7 June. Except where specified, chlorotic lesions were included in the data. After-infection and presymptom schedules showed little increase in percent leaves infected between 6 June and 28 July, while disease development on postsymptom treatments was steady, but significantly less than for unsprayed controls. Data for lesions per terminal, taken on 28 July, included an additional factor to correct for chlorotic lesions (Table 3). A comparison of total lesions per terminal with normal lesions per terminal showed that more lesions on bitertanol treated trees were chlorotic compared to CGA-64251 treatments.

Effect of Fungicides on Lesion Development and Isolation Recovery in 1980

Lesions from the 27 and 28 April infection periods were collected for scanning electron microscope examination on 21 May, 2 days after the second presymptom treatments on 19 May; on 18 May, 2 days after the first postsymptom treatments on 16 May; and on 25 May, 2 days after the second postsymptom treatments on 23 May.

The density of conidia in lesions from trees sprayed after lesions appeared (Figure 5C) was less than in lesions from unsprayed trees (Figure 5A). In addition, conidia in lesions from the presymptom treatments were ampulliform and appeared immature (Figure 5D), while conidia in lesions from unsprayed trees were mature in the lesion center (Figure 5B) with a narrow zone of ampulliform conidia

TABLE 3.--Control of scab on McIntosh apple with protective and postinfection eradicant treatments of CGA-64251 10% W 18.7 µg a.i./ml and bitertanol 50% W 299.6 µg a.i./ml applied in dilute sprays under orchard conditions in 1980.

Treatments	Number of Sprays	Incidence of Scab on Spurs[x] 22 May: Leaves Infected (%)	22 May: Lesions per Spur[y]	5 June: Leaves Infected (%)	5 June: Lesions per Spur[y]	Incidence of Scab on Terminals[x] 6 June: Leaves Infected (%)	6 June: Lesions per Terminal[y]	24 June: Leaves Infected (%)	24 June: Lesions per Terminal[y]	Incidence of Scab on Terminals[x] 28 July: Leaves Infected (%)	28 July: Total Lesions per Terminal[y]	28 July: Normal Lesions per Terminal[y]	Scab on Fruit 5 Sept: Fruit Infected (%)
Protective Treatments:													
7-day Schedules													
Bitertanol	15	0.0[z]	0.00[z]	1.0 a	0.05 a	1.5 ab	0.76 ab	0.8 a	0.29 a	0.5 a	0.18 a	0.0[z]	1.00 a
CGA-64251	15	0.0[z]	0.00[z]	0.0[z]	0.00[z]	0.2 a	0.05 a	0.2 a	0.22 b	0.3 a	0.05 a	0.0[z]	5.25 ab
14-day Schedules													
Bitertanol	8	1.5 a	0.16 a	5.2 a	0.48 ab	9.2 bc	11.66 abc	1.8 b	12.62 c	15.3 b	10.90 b	0.3 a	5.75 ab
CGA-64251	8	3.5 abc	0.37 ab	5.2 a	0.80 abc	24.5 de	29.20 d	25.8 bc	31.45 c	16.7 b	20.70 b	5.3 ab	12.75 abc
Postinfection Treatments:													
After-infection Schedules													
Bitertanol	7	0.5 a	0.03 a	1.2 a	0.05 a	5.5 abc	3.64 abc	9.8 b	6.89 c	19.7 bc	19.20 b	1.8 ab	5.00 ab
CGA-64251	7	5.2 ab	0.74 abc	5.5 a	0.46 ab	12.8 cd	13.20 bcd	15.2 bc	12.28 c	21.5 bcd	27.10 bc	15.1 b	13.25 bc
Presymptom schedules													
Bitertanol	6	16.8 d	2.27 cd	22.5 b	3.64 c	14.2 cd	21.94 cd	33.8 c	42.05 c	36.3 cd	61.70 bc	24.8 b	21.50 c
CGA-64251	6	16.0 cd	2.74 cd	20.0 b	2.47 bc	1.8 ab	0.38 ab	21.2 bc	20.52 c	27.3 bcd	46.90 bc	33.9 b	8.00 ab
Postsymptom schedules													
Bitertanol	6	13.5 bcd	1.79 bcd	20.2 b	2.05 bc	11.2 bc	12.71 bcd	24.2 bc	31.14 c	24.7 bcd	26.40 bc	7.2 ab	6.25 ab
CGA-64251	6	19.0 d	2.55 d	24.0 b	2.57 c	2.5 abc	1.02 abc	34.5 c	36.08 c	42.3 d	77.80 c	45.2 b	10.25 abc
Untreated (controls)	-	23.0 d	3.56 d	55.8 c	20.65 d	38.2 e	---	82.0 d	---	96.0 e	---	---	100.00 d

[x]Means followed by the same letter did not differ significantly (P = 0.05) using Duncan's Multiple Range test on arcsin-transformed percentage data and log transformed lesion data.

[y]The mean number of leaves per spur (or terminal) was 6.7, 5.3, 12.5, 16.8, and 19.1 on 22 May, 5 June, 6 June, 24 June, and 28 July, respectively. There was no significant difference between the mean number of leaves on different treatments for any of the dates.

[z]Data were not included in statistical analysis.

Figure 5.--Scanning electron micrographs of typical lesions and conidia of Venturia inaequalis from apple trees sprayed with bitertanol 50% W 299.6 µg a.i./ml and CGA-64251 10% W 18.7 µg a.i./ml.

(A) Lesion from unsprayed tree with dense sporulation (X390).

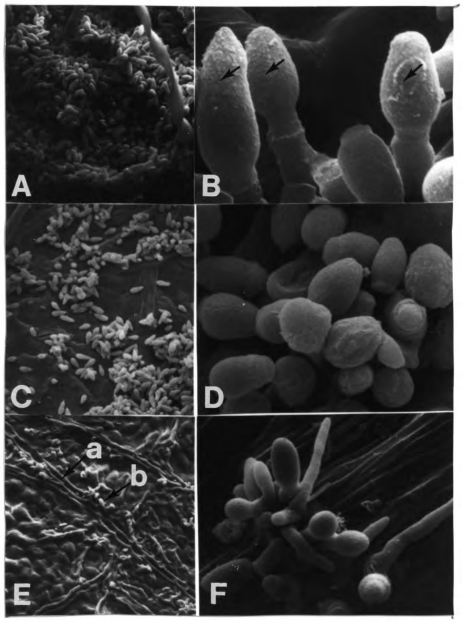
(B) Obpyriform conidia in lesion from unsprayed tree (arrow) (X4800).

(C) Reduced sporulation in a lesion sprayed 2 days earlier with bitertanol (X390).

(D) Ampulliform immature conidia in a lesion sprayed twice with bitertanol and sampled 2 days after the second spray (X2840).

(E) Subcuticular (a) and infrequent deformed surface growth (b) in a chlorotic lesion from a tree sprayed with CGA-64251 on a 14-day schedule (X390).

(F) Surface growth in a chlorotic lesion from a tree sprayed with bitertanol on a 14-day schedule (X2045).


A
B
C
D
a
b
E
F

at the lesion margins. Lesions taken from postsymptom sprayed trees, either after the first or second spray, had normal obpyriform spores in the lesion centers but a wider zone of ampulliform spores than lesions from unsprayed trees.

When lesions from pre- and postsymptom sprayed trees were rubbed across PDA, the conidia were difficult to remove and did not germinate, while large numbers of viable conidia were obtained from unsprayed lesions.

Chlorotic lesions on leaves exposed to the 13 and 17 May infection periods were taken on 10 June from the 14-day and postinfection schedules of CGA-64251 and bitertanol, and from presymptom and postsymptom treatments of bitertanol. Subcuticular fungal growth was visible in most chlorotic lesions (Figure 5E). Sporulation, with many ampulliform conidia, was observed in lesions from the after-infection schedules for both chemicals, but only scattered abnormal surface growth was observed for all other treatments (Figures 5E and 5F). The ratio of lesions with significant fungal growth to total number examined was 1/6 and 5/5 for the 14-day and after-infection treatments, respectively, of CGA-64251; and 3/6, 1/5, 6/7, and 5/5 for the 14-day, presymptom, after-infection, and postsymptom treatments, respectively, of bitertanol.

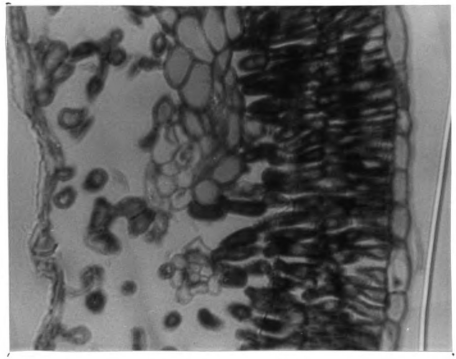
Isolations were attempted from chlorotic lesions collected 3 June from terminal leaves exposed to the infection periods of 13 and 17 May. The ratio of successful isolations of V. inaequalis to the total number of lesions examined were 5/11 (45.5%) for lesions from the 14-day schedule of CGA-64251 and 4/8 (50%) and

7/10 (70%) for lesions from the 14-day and postinfection treatments of bitertanol. The ratio of isolation was 11/11 (100%) for lesions from unsprayed leaves.

Effects of Tree Growth

Modified growth patterns were noted with both compounds in the 1979 and 1980 field studies. The elongation of lateral shoots was retarded and leaves were smaller, thicker, puckered, and darker green than leaves on unsprayed trees. The effects were more pronounced with CGA-64251 than bitertanol, and were most severe on trees treated with a 7-day schedule. Transmission electron microscope examination of leaf samples taken from trees sprayed on a 7-day schedule with CGA-64251 showed no abnormalities in cuticle structure or in organelles and internal cell structure. However, light microscope examination of several leaf sections revealed an increase in the number of palisade layers in leaves from sprayed trees (Figure 6B) compared to leaves from unsprayed trees (Figure 6A).

Figure 6A.--Cross-sections of apple leaves taken from unsprayed trees. Palisade cells are two to three layers deep.



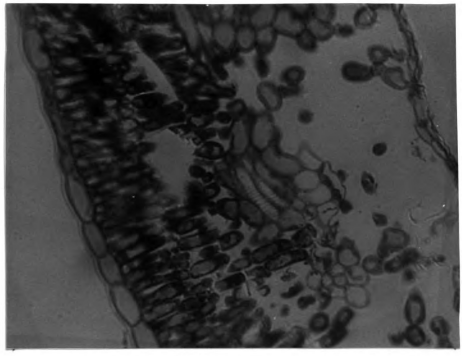
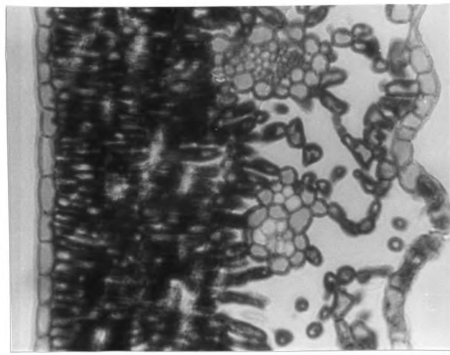
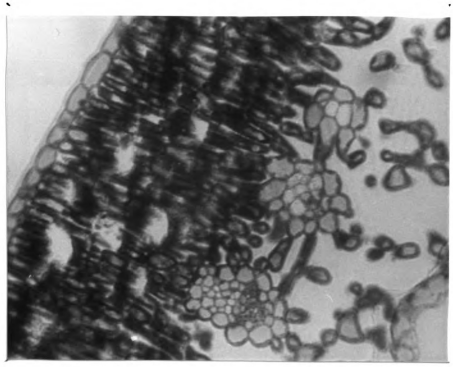


Figure 6B.--Cross-sections of apple leaves taken from trees sprayed with CGA-64251 on a 7-day schedule. Palisade cells are three or more layers deep.





DISCUSSION

Results from the greenhouse studies indicated that with CGA-64251, phenapronil, and high rates of bitertanol, infections were controlled when the chemicals were applied within 3 days after inoculation. Similar results were obtained in field trials with CGA-64251 and bitertanol. Therefore, all three compounds should be effective as after-infection controls in apple scab pest management schemes where dilute sprays are applied up to 3 days after the beginning of infection periods.

Field data from 1979 and 1980 indicate that CGA-64251 and bitertanol are capable of inactivating and controlling apple scab when applied several days after infection has occurred. If the compound is applied within 3 days after an infection period, one spray is sufficient to control infection from that infection period. However, if sprays are applied later in the infection period or after lesions are visible control is still possible but two sprays, 1 wk apart, must be applied.

In 1980 two infection period sequences were of particular importance; a heavy infection on 17 May when 3.02 cm of rain was recorded, and a series of four moderate and one light infection periods occurring in the first week of June. Both primary and secondary inoculum were present during these infection periods,

and many heavily-infected unsprayed trees were present in the orchard. As a result of high inoculum pressure and ideal weather for infection, many treatments developed chlorotic lesions, indicating partial control. These lesions failed to develop further, but did contribute to the lesion count data for these treatments.

Analysis of disease development on the after-infection treatments throughout the season indicated that disease was controlled for the primary infection periods in late April; however, the assumption of effective protective control for the heavy infection period in mid-May was probably not justified, and a certain amount of disease became established. This was, however, brought under control, and successfully controlled throughout the season, as shown by the lack of significant increase in disease for this schedule. While a disease increase was noted for the presymptom schedules between 6 June and 24 June, due primarily to chlorotic lesion development, control was also achieved on these treatments as shown by the lack of increase in percent leaves infected on 28 July. Disease increased steadily on postsymptom treatments. This is not surprising, as spray was not applied in these schedules until lesions were observed. An important feature of this increase is its controlled incremental nature, indicating that even though lesions were allowed to occur after each infection period, two sprays brought the disease under control each time. The scab which occurred on the presymptom treatments was probably the result of inaccuracy in estimating the number of days between infection and sporulation; for example, the first presymptom

spray was applied on 12 May and the first lesions were observed on 13 May.

Scanning electron micrographs showed a reduction in conidial density within sprayed lesions, and many conidia were immature. The percentage of immature forms was higher for treatments applied soon after an infection period. Various researchers (1, 3, 7, 10, 17) have reported reductions in conidia with postsymptom applications of benomyl and dodine, particularly if more than one spray was applied. Although attempts to germinate conidia from treated lesions were unsuccessful, isolation from some chlorotic lesions was possible. Thus, the action of these fungicides on subcuticular growth may be fungistatic, and failure to apply the second spray or to continue the spray program into the season could result in renewed activity of previously suppressed lesions. This failure to affect subcuticular growth has been previously reported for dodine, captan, zineb, and lime sulfur (2), however, the possibility that a cumulative effect from repeated spraying through the season is likely. The possibility that the fungus in suppressed lesions may be capable of ascocarp production during the winter, and thus contribute to primary inoculum in the following year, should be examined.

LITERATURE CITED

1. Albert, J. J., and F. H. Lewis. 1962. Effect of repeated applications of dodine and of captan on apple scab foliage lesions. Plant Dis. Rep. 46:163-167.

2. Albert, J. J., and J. W. Heuberger. 1961. Apple scab I. Effect of one application of fungicides on leaf lesions on previously unsprayed trees. Plant Dis. Rep. 45: 759-763.

3. Alexander, S. A., and F. H. Lewis. 1975. Reduction of apple scab fungus inoculum with fungicides. Plant Dis. Rep. 59:890-894.

4. Buchel, K. H., W. Kramer, W. Meiser, W. Brandes, P. E. Frahberger, and H. Kaspers. 1979. Chemistry and properties of phenyl-triazole-methanes, a highly active class of new azole fungicides. Abstract 475 in Abstracts of Papers, IXth Int. Congr. of Plant Protection, Washington, D.C.

5. Carley, H. E. 1979. A new multiple use fungicide: α-butyl-α-phenyl-1H-imidazole-1-propanenitrile. Phytopathology 69:1023.

6. Fuchs, A., and W. Ost. 1976. Translocation, distribution and metabolism of triforine in plants. Archives of Environ. Contam. and Toxicol. 4:30-43.

7. Heuberger, J. W., and R. K. Jones. 1962. Apple scab II. Effect of serial applications of fungicides on leaf lesions on previously unsprayed trees. Plant Dis. Rep. 46:159-162.

8. Hoch, H. C., and M. Szkolnik. 1979. Viability of Venturia inaequalis in chlorotic flecks resulting from fungicide application to infected Malus leaves. Phytopathology 69:456-462.

9. Jones, A. L., S. L. Lillevik, P. D. Fisher, and T. C. Stebbins. 1980. A microcomputer-based instrument to predict primary apple scab infection periods. Plant Dis. 64: 69-72.

10. Jones, R. K., J. W. Heuberger, and J. D. Bates. 1963. Apple scab III. Effect of serial applications of fungicides on leaf lesions on previously unsprayed trees--inhibition of conidial germination, removal (suppression) of the organism, and subsequent development of late terminal infection. Plant Dis. Rep. 47:420-424.

11. Mills, W. D., and A. A. LaPlante. 1951. Control of diseases and insects in the orchard. N.Y. Agric. Exp. Stn. (Ithaca) Ext. Bull. 711, pp. 18-22.

12. Pearson, R. C., M. Szkolnik, and F. W. Meyer. 1978. Suppression of cedar apple rust pycnia on apple leaves following postinfection applications of fenarimol and triforine. Phytopathology 68:1805-1809.

13. Preece, T. F. 1959. A staining method for the study of apple scab infections. Plant Pathol. 8:127-129.

14. Prusky, D., Benjamin Jacoby, and A. Dinoor. 1979. Is the accumulation of a systemic fungicide at the infection sight related to its eradicative action? Pest. Biochem. and Phys. 12:75-78.

15. Ragsdale, Nancy N. 1975. Specific effects of triarimol on sterol biosynthesis in Ustilago maydis. Biochemica et Biophysica Acta 380:81-96.

16. Staub, T., F. Schwinn, and P. Urech. 1979. CGA-64251, a new broad-spectrum fungicide. Phytopathology 69:1046.

17. Szkolnik, M., J. R. Nevill, and L. M. Henecke. 1973. Fungicidal inhibition of production of new conidia from established foliar apple scab lesions. Phytopathology 63:208.

18. Tehon, L. R., and G. L. Stout. 1930. Epidemic diseases of fruit trees in Illinois: 1922-1928. Ill. Nat. Hist. Surv. Bull. 18:415-502.

19. Tuite, John. 1969. Plant pathological methods: fungi and bacteria. Burgess Publishing Co., Minneapolis, MN., 239 pp.

PART II

VOLATILITY AND SYSTEMIC PROPERTIES IN APPLE OF CGA-64251, BITERTANOL, AND PHENAPRONIL FUNGICIDES

INTRODUCTION

Before a fungicide can be effectively incorporated into a pest management program, not only its disease control abilities, but also the characteristics of its movement and localization within the plant must be determined. The ability of a systemic fungicide to enter the plant, and the extent and speed of its movement after entry, determine the application conditions which are optimally effective and the level and type of control which can be expected. Solel and Edgington (4) studied the transcuticular movement of fungicides using isolated apple cuticles, and Edgington et al. (2) have used washings of treated leaves to determine the amount and rate of uptake. Also, the volatility of a compound may seriously affect its control characteristics. In the case of fungicides for the control of apple scab, volatilization in the orchard after application could result in a reduction in the period of effective protective control of disease and a reduction in the amount of fungicide available for uptake by the plant. When used in an enclosed environment, such as the greenhouse, however, an increase in control may be noted (1, 3, 5).

In this study, the uptake and distribution properties in apple of three experimental compounds which are characteristics of a family of ergosterol inhibiting fungicides presently being

developed, were examined by using leaf washings and by oxidation of the plant parts after application of radioactive labeled compounds. In addition, autoradiographs were made to further visualize distribution patterns.

MATERIALS AND METHODS

Volatility of Fungicides

A 10 µl droplet of radioactive fungicide was applied with a syringe, fitted with a 10 µl calibrated disposable capillary pipet, to round microscope cover glasses held in the dark at 20 C and at a relative humidity of 5%. At various times after application, five cover glasses for each fungicide were each dropped into 15 ml scintillation fluid (Aqueous Counting Scintillant, Amersham Corp., Arlington Heights, IL 60005), shaken thoroughly, and counted in a Searle, Model 6868 ISOCAP/300 liquid scintillation counter (Searle Analytic Inc., Des Plaines, IL 60018). Each sample was counted for 10 min, and disintegrations per minute (DPM) calculated from a quenched external standard curve.

Uptake of Fungicides

Thirty-six single-shooted McIntosh apple (Malus pumila Mill.) trees growing in pots in the greenhouse were used. Ten 10 µl droplets of radioactive fungicide were applied to the youngest fully expanded leaf of each plant with a calibrated capillary pipet. At various times after application, the shoots and leaves were cut from three trees for analysis. Each cut was made with a new razor blade to avoid contamination. The two leaves below and the two leaves above the leaf to which fungicide was applied, the growing

tip cluster, and the fungicide treated leaf were each oxidized at 900 C in an OX-200 Biological Material Oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ 97642). The $^{14}CO_2$ was trapped in a 2:1 mixture of Permafluor V Scintillant and Carbosorb II carbon dioxide absorber (Packard Instrument Co., Downers Grove, IL 60515) and each sample was counted in the scintillation counter. Before oxidizing, the fungicide treated leaf was washed in 10 ml of 95% ethanol to remove fungicide remaining on the leaf surface. In a preliminary study, it was determined that more than 90% of the radioactivity was removed in the first wash and very little fungicide was removed by additional washings. The wash was added to 10 ml scintillation fluid and counted. Zero-time values were determined by applying ten 10 µl droplets to leafsized pieces of analytical filter paper, oxidizing, and counting.

Autoradiography

Nine equal-sized single-shooted, McIntosh apple trees growing in pots in the greenhouse were separated into three groups, with each group of three trees receiving a different compound. Three 10 µl droplets of radioactive fungicide were applied with a calibrated capillary pipet to the youngest fully expanded leaf of each plant. The plants were held in the greenhouse for 7 days at about 18 to 25 C, after which the shoots were removed, placed on Fuji No-Screen X-ray film, and pressed in a plant press. The pressed plants were stored at -10 C for 21 days and the films developed in Kodak Liquid X-ray Developer (number 146 5335).

RESULTS

Volatility of Fungicides

Radioactive bitertanol ((UL-byphenyl-^{14}C)-Baycor, specific activity 41.72 μCi/mg) was supplied by Mobay Chemical Corp., Kansas City, MO 64120. Radioactive CGA-64521 ((U-triazole ring-^{14}C)-CGA-64251, specific activity 61.1 μCi/mg) was supplied by Ciba-Geigy Corp., Greensboro, NC 27409. Radioactive phenapronil ((ring-^{14}C)-Sisthane, specific activity 8.93 μl Ci/mg) was supplied by Rohm and Haas Corp., Philadelphia, PA 19105. All fungicides were initially dissolved in 1 ml of ethanol and diluted to 10X final test strength (6170 μg active ingredient (a.i.)/ml, 2996 μg a.i./ml, and 187 μg a.i./ml for phenapronil, bitertanol, and CGA-64251, respectively) with distilled water. Dilutions to test strength were made by adding portions of this stock solution to distilled water.

Cover glasses with fungicide were placed in scintillation vials at 0.5, 1, 2, 4, 8, 24, 48, 72, and 120 hr after application. Phenapronil and bitertanol samples showed no reduction in radioactivity in 120 hr (Figure 1). When these data were subjected to regression analysis, the best line fit to each data set had a slope not significantly different from zero (P=0.01). The radioactive CGA-64251 dropped by about 50% in 72 hr, and a multiple regression analysis of the data yielded the equation:

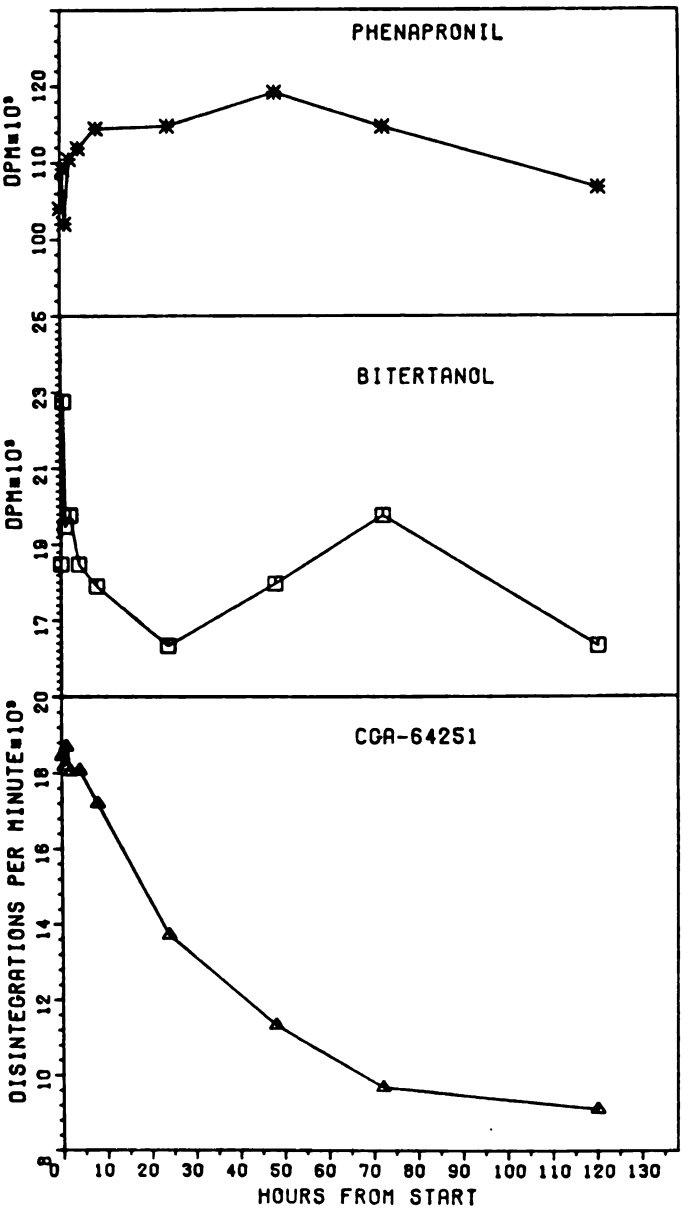


Figure 1.--Radioactive fungicide remaining on cover glasses at various times after application.

$$Y = Ke^{(-0.0062\ X)} \qquad (r^2 = 0.8216)$$

where K is the amount of radioactivity applied at time zero in DPM, X is hours after application, e is the irrational constant 2.71828, and Y is the amount of radioactivity remaining at time X.

Uptake of Fungicides

Radioactive phenapronil (617 µg a.i./ml), bitertanol (299.6 µg a.i./ml), and CGA-64251 (18.7 µg a.i./ml) were applied to apple leaves and samples were taken at 0.5, 1, 3, and 7 days for analysis. Radioactivity was detected in all samples. The average amount of fungicide that moved out of the treated leaf was 5.7, 27, and 2.3% for CGA-64251, bitertanol, and phenapronil, respectively. All chemicals moved both upward and downward, and less radioactivity was detected, after 7 days, in the leaves immediately above or below the treated leaf than in the uppermost leaves or in the second leaf below the treated leaf (Figure 2). At 12 hr, 90% of the radioactive CGA-64251 had entered the leaf, after which equilibrium was maintained (Figure 3A). The other fungicides moved into the leaves more slowly (Figures 3B and 3C). Equilibrium was reached at 24 hr for bitertanol and within 3 days for phenapronil, with about 40 and 60% of the radioactivity, respectively, crossing the cuticle.

Autoradiography

Radioactive phenapronil (6170 µg a.i./ml), bitertanol (2996 µg a.i./ml), and CGA-64251 (187 µg a.i./ml) were applied

APPLE LEAF POSITION	% OF MOBILE FRACTION		
	BITERTANOL	CGA-64251	PHENAPRONIL
	26	24	37
	40	17	19
	3	16	5
	TREATED LEAF		
	6	12	3
	25	31	36

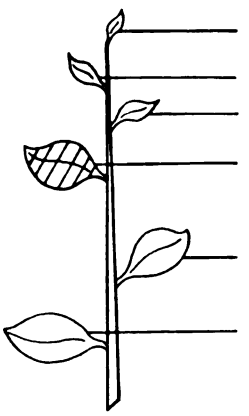


Figure 2.--Distribution of the portion of radioactive fungicide which had moved out of treated leaves, 7 days after application.

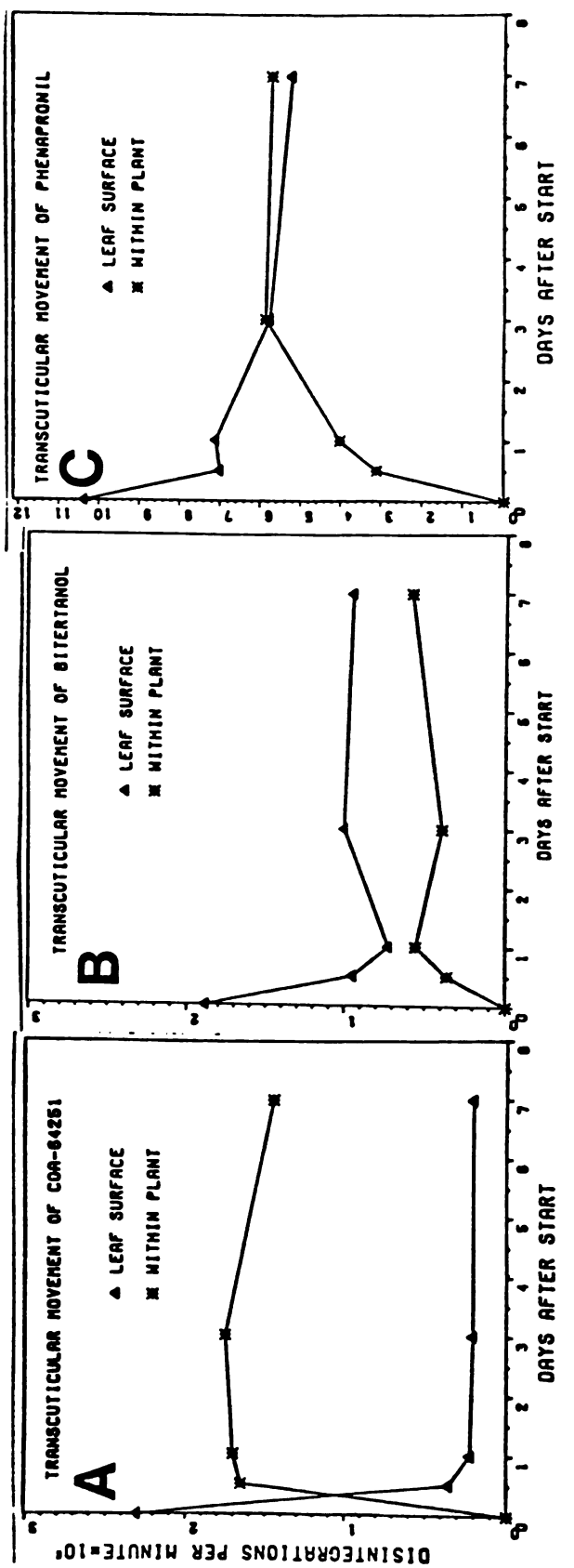


Figure 3.--Transcuticular movement of radioactive fungicide into apple leaves.

to plants. Autoradiographs confirmed the results of the uptake studies, but showed distinct differences in distribution patterns between the three fungicides. A large amount of labeled CGA-64251 and phenapronil was distributed throughout the leaves to which they were applied (Figures 4A and 4B). Accumulation of labeled chemical was also noted in the shoots and, with phenapronil and CGA-64251, labeled chemical was observed in the growing tip and the lower leaves. By contrast, most of the labeled bitertanol that remained in the treated leaf did not move from the spots where it was applied (Figure 4C). There was also accumulation in the major veins, but little or no movement into the interveinal areas. Small amounts of labeled chemical were also noted in leaves above and below the treated leaf.

Figure 4A.--X-ray film autoradiograph showing the pattern of movement and distribution of radioactive fungicides in apple shoots 7 days after application of CGA-64251 to a single leaf (the darkest leaf).

Figure 4B.--X-ray film autoradiograph showing the pattern of movement and distribution of radioactive fungicides in apple shoots 7 days after application of phenapronil to a single leaf (the darkest leaf).

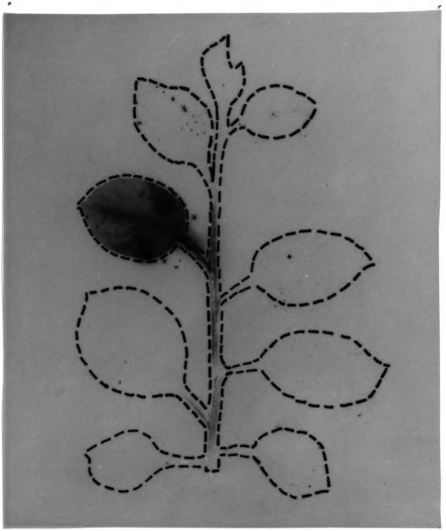
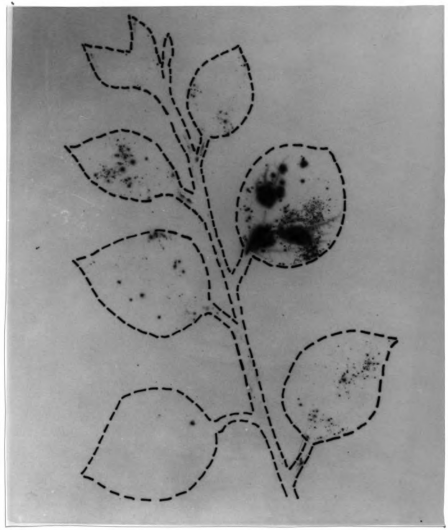


Figure 4C.--X-ray film autoradiograph showing the pattern of movement and distribution of radioactive fungicides in apple shoots 7 days after application of bitertanol to a single leaf (the darkest leaf).



DISCUSSION

Analysis of the potential for volatilization of these three fungicides from cover glasses indicated that a significant portion of CGA-64251 is volatilized within 24 hr after application, which supports previous research by Szkolnik (5). In orchard applications this may not be of concern, as uptake studies showed that a very high percentage of the compound crosses the cuticle and enters the leaf within 12 hr after application; however, volatilization may be an important source of control in greenhouse situations. In addition, the ability to enter the plant quickly reduces the length of time the chemical is exposed to the possibility of being washed from the plant by rain. The entry rates of bitertanol and phenapronil were somewhat slower, and thus they would be exposed on the leaf surface for a longer time; however, they showed no volatilization within 120 hr.

The movement and distribution of the fungicides varied considerably between plants. However, a pattern was evident. Fungicide moved out of the leaf to which it was applied much more rapidly if the plant was actively growing. If the shoot failed to elongate, or elongated only slightly, translocation was slow and the amount moved was less. The highest concentration of the mobile fraction seemed to be associated with the leaves closest to the

fungicide treated leaf at 3 days, but at 7 days, the primary sites of concentration were the growing tip and the lower leaves on the stem.

Autoradiographs showed a pattern of distribution similar to the scintillation studies. Only a very small fraction of the applied fungicide is moved out of the treated leaf and into the growing tip. This may not be enough fungicide to achieve control of scab on the new tissue, and this possibility should be investigated further.

LITERATURE CITED

1. Clark, Terence, Derek R. Clifford, Adrian H. B. Deas, Peter Gendle, and David A. M. Watkins. 1978. Photolysis, metabolism and other factors influencing the performance of triadimefon as a powdery mildew fungicide. Pesticide Science 9:497-506.

2. Edgington, L. V., P. P. Q. de Wildt, K. Jacques, and J. Psutka. 1976. The study of transcuticular movement of fungicides, in Proceedings: Apple and pear scab workshop, pp. 32-35, A. L. Jones and J. D. Gilpatrick, eds., N.Y. Agric. Exp. Stn., Special Report 28, 38 pp.

3. Scheinpflug, H., and V. Paul. 1977. On the mode of action of triadimefon. Neth. J. Pl. Path. 83 (suppl. 1):105-111.

4. Solel, Z., and L. V. Edgington. 1973. Transcuticular movement of fungicides. Phytopathology 63:505-510.

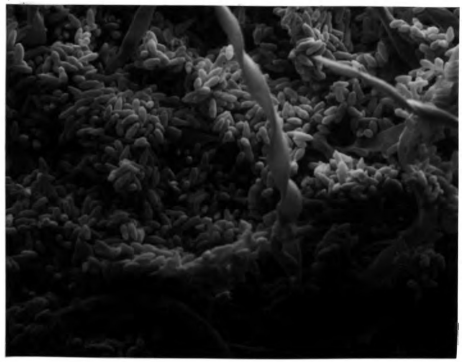
5. Szkolnik, M. 1980. Control of apple powdery mildew by vapor from a new triazole fungicide. Phytopathology 70:469.

APPENDICES

APPENDIX A

SCANNING ELECTRON MICROGRAPHS OF LESIONS SPRAYED WITH CGA-64251 OR BITERTANOL

Figure A1.--Lesion (top, X390) of *Venturia inaequalis* from untreated trees, showing dense sporulation and normal mature conidia (bottom, X4800).



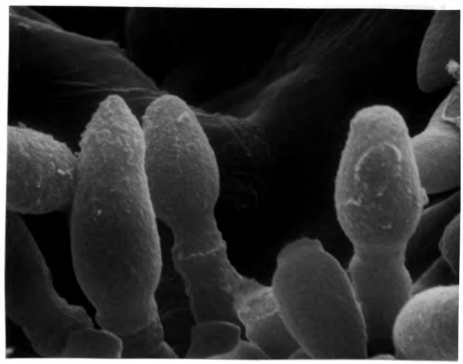
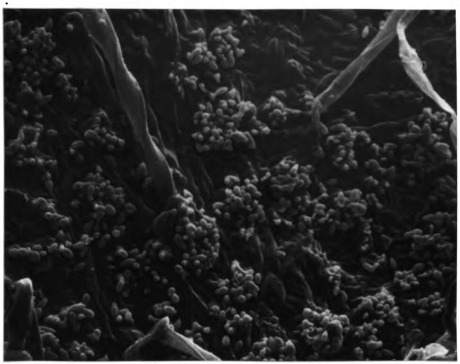


Figure A2.--Lesion (top, X390) of Venturia inaequalis from presymptom treatment of CGA-64251, taken 2 days after second spray was applied. Conidial density is less than for unsprayed lesions and conidia (bottom, X1390) are immature and ampulliform.



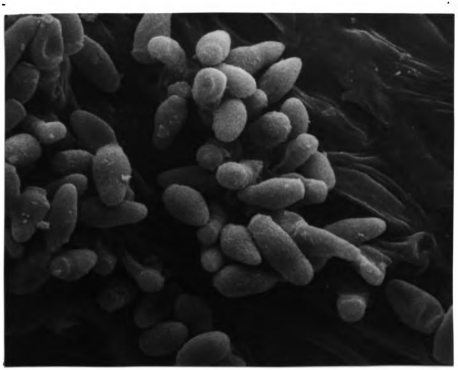
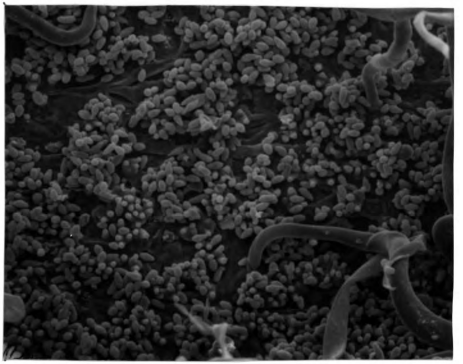


Figure A3.--Lesion (top, X390) of Venturia inaequalis from presymptom treatment of bitertanol, taken 2 days after second spray was applied. Conidial density is less than for unsprayed lesions and many conidia throughout the lesion (bottom, X2840) are immature and ampulliform.



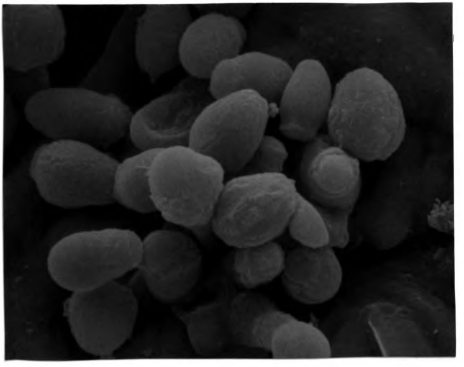
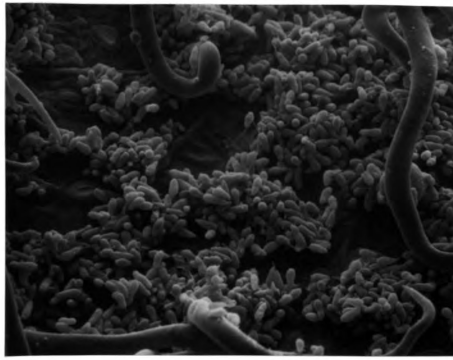


Figure A4.--Lesion (top, X440) of Venturia inaequalis from postsymptom treatment of CGA-64251, taken 2 days after first spray was applied. Conidial density is less than for unsprayed lesions with many immature conidia at the lesion margins. Conidia at lesion center are normal and mature (bottom, X950).



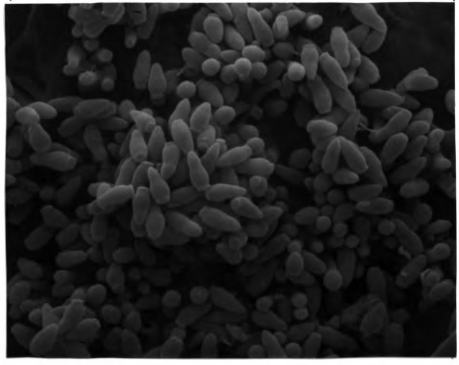
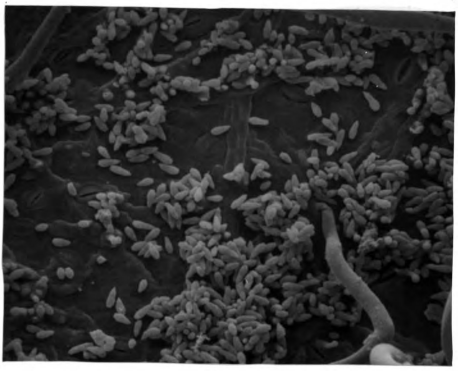


Figure A5.--Lesion (top, X390) of Venturia inaequalis from postsymptom treatment of bitertanol, taken 2 days after first spray was applied. Conidial density is less than for unsprayed lesions with many immature conidia at the lesion margins. Conidia at lesion center are normal and mature (bottom, X950).



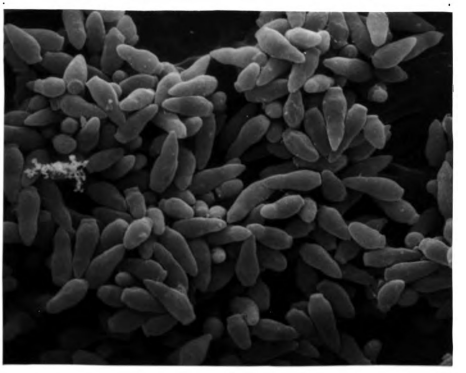
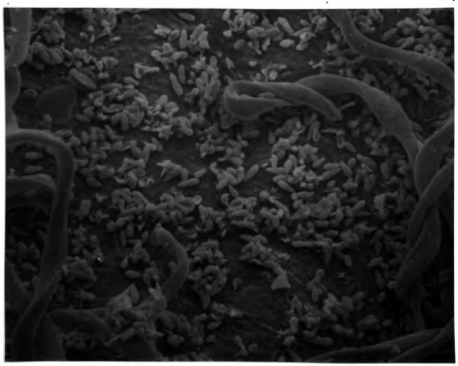


Figure A6.--Lesion (top, X390) of Venturia inaequalis from postsymptom treatment of CGA-64251, taken 2 days after second spray was applied. Conidial density is less than for unsprayed lesions with many immature conidia at the lesion margins. Conidia at lesion center are normal and mature (bottom, X4800).



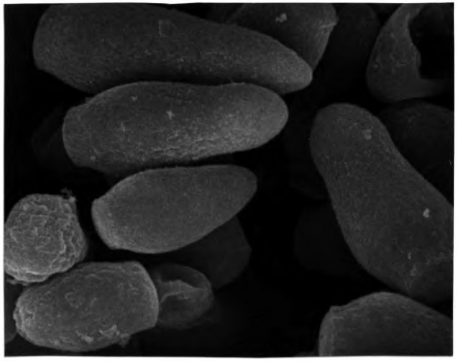
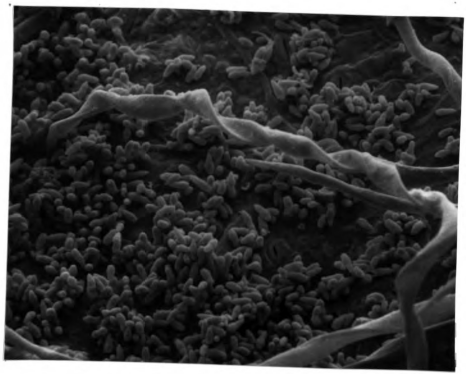
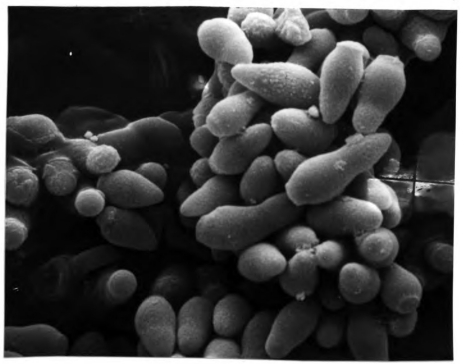


Figure A7.--Lesion (top, X390) of Venturia inaequalis from postsymptom treatment of bitertanol, taken 2 days after second spray was applied. Conidial density is less than for unsprayed lesions with many immature conidia at the lesion margins. Conidia at lesion center are normal and mature (bottom, X1890).





APPENDIX B

SCANNING ELECTRON MICROGRAPHS OF CHLOROTIC LESIONS SPRAYED WITH CGA-64251 OR BITERTANOL

Figure B1.--Chlorotic lesion (top, X390) and deformed surface growth (bottom, X3040) of Venturia inaequalis from 14-day treatment of CGA-64251, taken on 10 June. Subcuticular growth is evident in the lesion center.

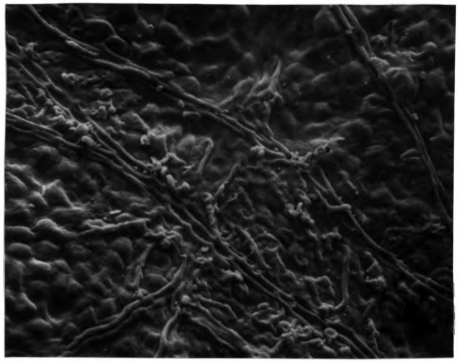
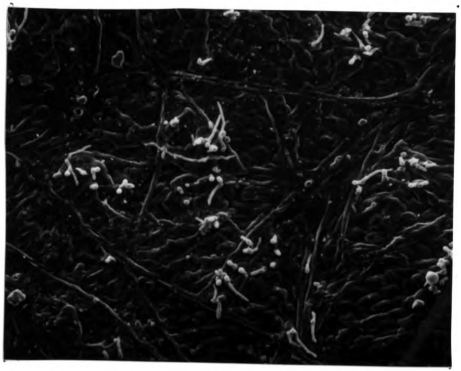


Figure B2.--Chlorotic lesion (top, X270) of Venturia inaequalis from 14-day treatment of bitertanol, taken 10 June. Most conidia are ampulliform and immature (bottom, X2045).



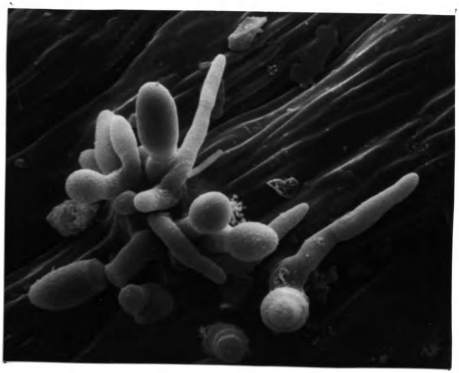


Figure B3.--Chlorotic lesion (top, X390) of Venturia inaequalis from after-infection treatment of CGA-64251, taken 10 June. Most conidia are ampulliform and immature (bottom, X2890).

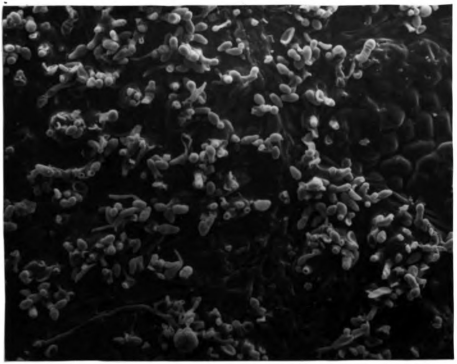
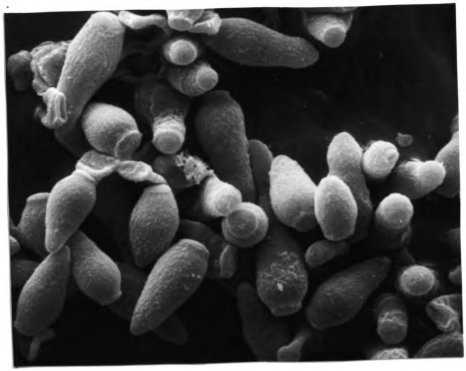
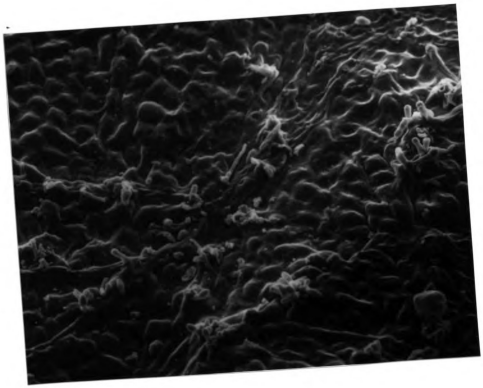


Figure B4.--Chlorotic lesion (top, X270) of Venturia inaequalis from after-infection treatment of bitertanol, taken 10 June. About 50% of conidia (bottom, X2060) are ampulliform and immature.





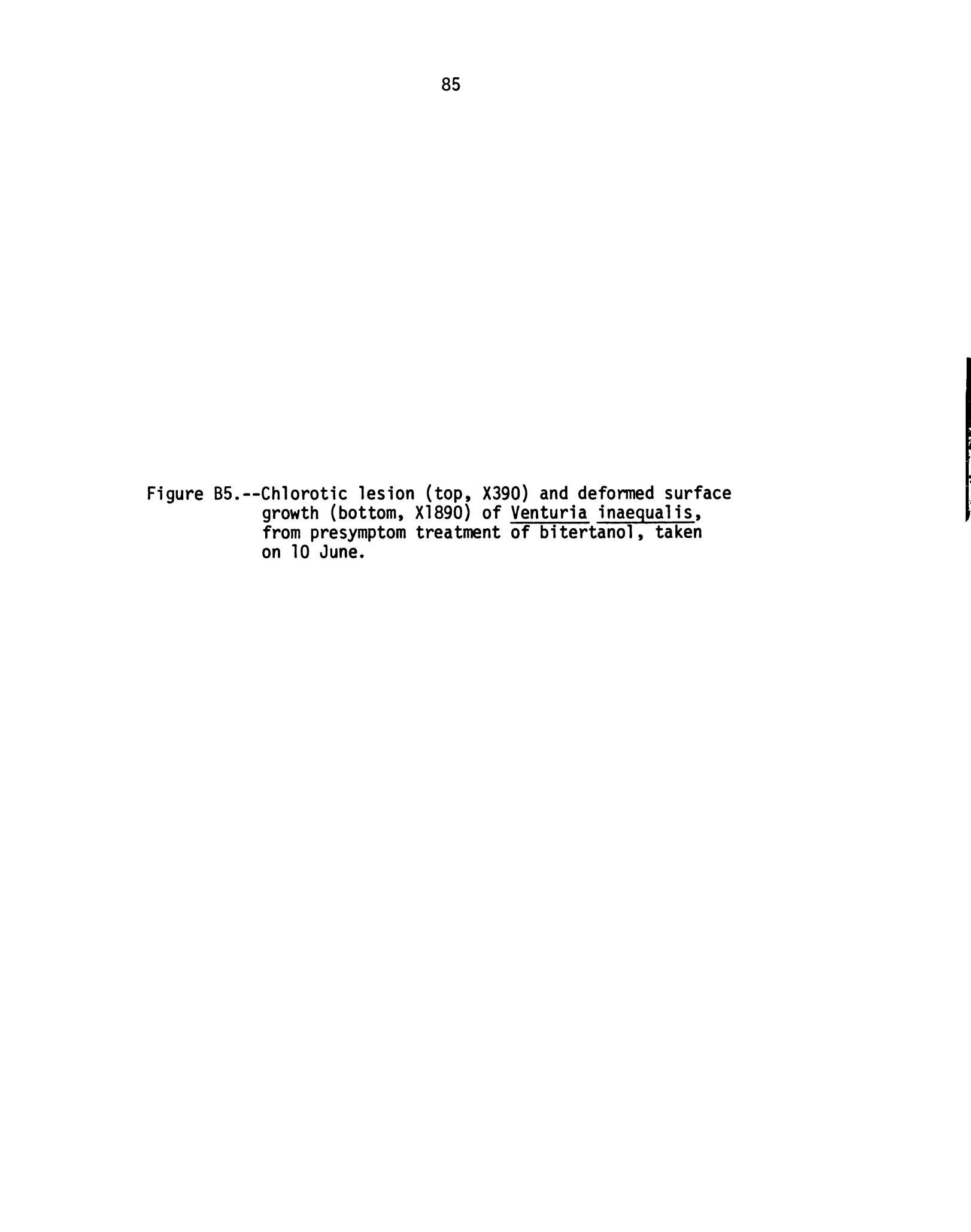
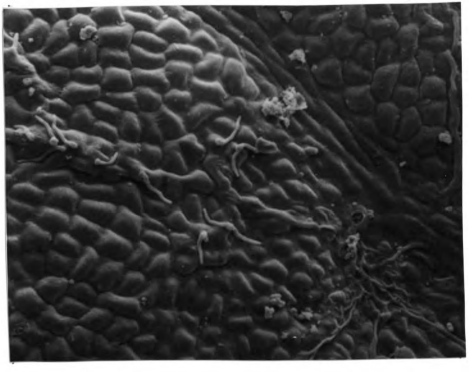


Figure B5.--Chlorotic lesion (top, X390) and deformed surface growth (bottom, X1890) of Venturia inaequalis, from presymptom treatment of bitertanol, taken on 10 June.

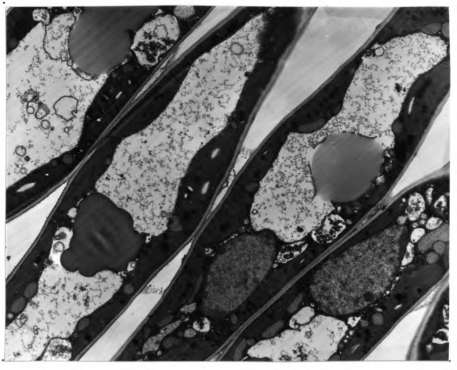
Figure B6.--Chlorotic lesion (top, X400) of Venturia inaequalis from postsymptom treatment of bitertanol, taken 10 June. Some subcuticular growth is evident at the lesion center. Sparse surface growth is abnormal and a few immature conidia are present (bottom, X3040).



APPENDIX C

TRANSMISSION ELECTRON MICROGRAPHS OF PALISADE CELLS OF APPLE

Figure C1.--Transmission electron micrographs of longitudinal section of apple palisade cells taken from leaves of 7-day schedule of CGA-64251. No membrane or organelle abnormalities are evident.



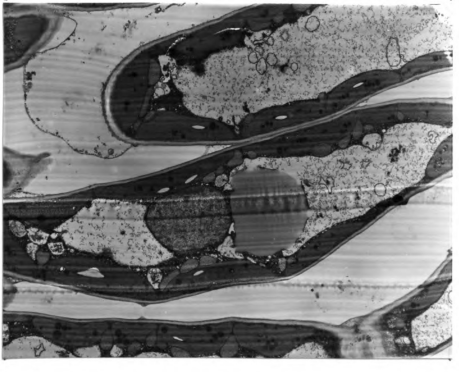


Figure C2.--Transmission electron micrographs of longitudinal section of apple palisade cells taken from leaves of unsprayed trees.