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Title: EFFECTS OF VOLATILE FUNGICIDES ON SPHAEROTHECA PANNOSA

ON ROSE   Redacted for privacy

Abstract approved:

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Sphaerotheca pannosa var. rosae was controlled in greenhouses
ranging in volume from 1886 m^3 to 4946 m^3 and in the laboratory by
heating the fungicides nuarimol and fenapanil at rates approximating
those used in conventional spray treatments. Bupirimate was effec-
tive when heated in laboratory tests. Fungicide volatilty and
efficacy was dependent on heating temperature and duration. Nuarimol
was ineffective as a fumigant at ambient temperature and 100% effec-
tive when volatilized by heating to 105°C. At 105°C bupirimate gave
no greenhouse control and 73% control in growth chambers, but control
increased to 100% when heated to 185°C. Increasing nuarimol volatili-
zation from two hr to four hr improved greenhouse control from 82% to
99%. No phytotoxicity was observed in any treatments. Foliage and
flower quality were excellent due to lack of spray residues. Nuarimol
fumigation controlled powdery mildew up to six mo after the last
treatment in commercial greenhouses. Glass, plastic and fiberglass
were shown to sorb nuarimol during fumigation and later release its
vapors.

All three fungicides rapidly killed one-day-old powdery mildew colonies on detached rose leaf tissue but only bupirimate prevented conidia germination. A few mature colonies remained alive after nuarimol treatment but none developed to sporulation. One-day-old colonies on detached leaf tissue were killed after fumigation with 1.8 mg a.i./m^3 nuarimol for two hr. Colonies developing from untreated conidia transferred to treated leaf tissue (1.8 mg a.i./m^3; two hr) were also killed. It was necessary to treat conidia with 10 mg a.i./m^3 for six hr before resulting colonies on untreated leaves were killed.

Attempts to induce the sexual stage of S. pannosa were unsuccessful. Nine monoconidial isolates paired in all combinations and all nine isolates combined failed to produce ascocarps on plants in greenhouse isolation chambers. Varying fertility, daylength, temperature and plant growth hormone treatments did not result in sexual stage production.

EFFECTS OF VOLATILE FUNGICIDES ON
SPHAEROTHECA PANNOSA ON ROSE

by

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INTRODUCTION

Powdery mildew of rose caused by the fungus Sphaerotheca
pannosa var. rosae (Wall. ex Fr.) Lev. is widespread and the most
serious disease faced by the cut rose producer. Control in the
greenhouse has been accomplished primarily through the use of
protective or eradicative sprays (19). Although fair to good
control can be achieved under most greenhouse conditions, spraying
is labor intensive. When greenhouse conditions are optimum for
powdery mildew development it becomes difficult for growers to
maintain adequate disease control to produce marketable blossoms,
because relatively few fungal colonies greatly reduce the aesthetic
value of the product.

Attempts have been made for many years to control rose powdery
mildew and other diseases of glasshouse crops through the use of
volatile properties of fungicides. Although the use of sulfur for
plant disease control dates back to 1846 (6), it was in 1852 that
Bergman reportedly sprinkled sulfur on moistened hot water pipes
in the greenhouse for grape powdery mildew control (3). Since
that time the volatile properties of sulfur have been the subject
of several studies (44,45,62) and this method is still in use
today in many commercial rose greenhouses (10). However, sulfur
provides only marginal control and is often erratic. The optimum
greenhouse temperature for control with sulfur fumes is 21-22°C

with little activity below 16°C and a high probability of phyto-
toxicity at temperatures above 30°C.

There have been a number of attempts to control diseases of
glasshouse crops using fungicides as smokes, but these have been
unsatisfactory because of unsightly residues, poor disease control
or phytotoxicity. McKeen (46), using Karathane as a smoke from a
pyrotechnic bomb, obtained good control of cucumber powdery mildew
in the greenhouse, but no data were presented, the treatment caused
injury and left a residue on the plants. Berry-Smith (4) controlled
Botrytis and powdery mildew on glasshouse grapes using tetrachloro-
nitrobenzene in a smoke generator, but no data were given. Smoke
generators made by mixing eight individual fungicides with potassium
chlorate, lactose and clay and primed with a layer of lactose and
potassium nitrate were tested by Lockhart and Eaves (39,40). The
generators were ignited with an electric heating element, but as
much as 98% loss of the toxicant occurred due to thermal decomposi-
tion. In addition, the smoke particles collected as a dust primarily
on horizontal surfaces resulting in lack of uniform control. Phyto-
toxicity occurred when the dosage was adequate for disease control.

Turner and Lamont (58) found that chlorothalonil sublimes when
heated and settles as a fine dust upon cooling. They tested the
chemical as a dust produced by subliming in a sulfur vaporizer
heated to 300°C or in pyrotechnic bombs. There was no phytotoxicity
and tomato plants were protected from infection by Alternaria solani
as were beans from Erysiphe polygoni. The residue from the smoke
bomb was considered objectionable and injury occurred on rose and

begonia blossoms from both methods. Due to the high temperatures generated in the smoke bomb they felt there was a considerable amount of thermal decomposition, although it was not measured. The temperature in the sulfur vaporizer could be maintained at 300°C, well below the thermal decomposition point of chlorothalonil. Commercial preparations of chlorothalonil are currently marketed principally for the control of Botrytis cinerea on greenhouse crops.

According to Lukens (41), fungi contact fungicides in the vapor state or as solutes, and several organic fungicides with very low vapor pressures migrate as vapors to the fungus from residues of dusts and sprays. Hislop (31) concluded that all fungicides with activity against the powdery mildews release fungicidal vapors since free water is not necessary for these fungi to germinate and develop. Therefore, no liquid medium is present through which the fungicide could migrate to reach the fungus as a solute. Coyier and Picchi (15) found that the volatility of fenarimol was sufficient to greatly reduce conidial germination and stop mycelial development of rose powdery mildew on detached leaves when incubated together in small chambers.

Vapor pressures of both liquids and solids increase as temperature increases, permitting a greater concentration of the liquid to volatilize into the vapor phase. It appeared reasonable that if the fungicide temperature was increased, its vapor pressure would increase correspondingly to volatilize the toxicant, diffuse and contact the pathogen in quantities sufficient for disease control. There have been no reports of attempts to control any disease in

this manner.

Acquired resistance to fungicides has become an increasingly important consideration in the chemotherapy of plant diseases primarily due to the introduction in recent years of many systemic fungicides which exert a greater specific selection pressure on target organisms than have the copper and dithiocarbamate fungicides in the past. Resistance may be the result of physiological adaptation in which case sensitivity quickly returns when the organism is no longer exposed to the toxicant. Genetic resistance is more stable (17,18). Many fungi have a propensity for genetic mutation, but such mutants are usually not as well adapted as wild types. When specific selection pressure is exerted by certain fungicides, mutants that would normally not survive or not become a significant part of the population may be capable of multiplication in the absence of sensitive wild types (17,18,26). Benzimidazole and thiophanate fungicides radically alter the stability of diploid strains of Aspergillus nidulans (30,35). Mutagenesis by certain fungicides could also cause the selection of resistant fungal populations, although I am unaware of data to support this concept under field conditions.

An understanding of the mode of action of the newer fungicides, the likelihood of the development of resistance in target organisms and the origin of such resistance is paramount in avoiding fungicide resistance. Because the powdery mildews are obligate parasites their genetics are more difficult to examine than are those of facultative parasites. In rose powdery mildew, the sexual stage

appears erratically and its role in the life cycle and the origin

of genetic specialization is unknown. However, ascocarps develop

with some regularity on hypanthia, pedicels, and to a lesser extent

on stems near thorns in the variety Dwarf Crimson Rambler. Coyier

(11) observed ascocarp development on Rosa viginiana leaves both in

the field and in the greenhouse. Price (48) found ascocarps on only

32 cultivars in a survey of 741 rose species and cultivars conducted

from 1966-1968. They were found embedded in the pannose mycelium

around the thorns, stems and hypanthia of the blooms primarily on

ramblers, climbers and old shrub roses. Several investigators

suggested that alternating temperature (1,5,9) and host senescence

(27,33) induced ascocarp formation in some mildew species. However,

Yarwood (61) found that Erysiphe cichoracearum DC. ex Merat was

heterothallic on sunflower and required 2 isolates for perithecia

formation. Old leaves, low host nutrition, dry atmosphere or low

temperature were not required. Schnathorst (51) demonstrated hetero-

thallism in E. cichoracearum on lettuce with continuous light and

constant temperature using conidial isolates taken from areas on

lettuce leaves where perithecia were abundant. Powers and Moseman

(47) reported heterothallism in Erysiphe graminis tritici Em.

Marchal using isolates from ascospores. Coyier (13) found Podosphaera

leucotricha (Ell. & Ev.) Salm. on apple to be heterothallic using

isolates of single conidia and maintaining paired cultures at 21-25°C

and 12 hours light. Smith (56) reported increased ascocarp production

in E. polygoni DC under long daylength (16 hr) and temperature between

10 and 20°C, but concluded that any irregularity in ascocarp formation

in the field is due to absence of the necessary mating-types rather than to an unfavorable environment or nutritive condition of the host.

Coyier (14) observed increased ascocarp production on apple shoots naturally infected with Podosphaera leucotricha after exogenously applying 10 ppm indole-3-butyric acid (IBA) in lanolin paste. The literature is extensive regarding the interrelationship of plant pathogens and plant growth hormones, but there has been no mention of a plant growth hormone effect on the sexual stage development of a fungal pathogen. Several reviews have appeared covering the relationship between plant pathogens and plant growth hormones (16,23, 28,29,52,53,59,60).

The purpose of this study was to (a) determine whether the volatility of fungicides could be increased sufficiently by heating to control rose powdery mildew in enclosed chambers and greenhouses, (b) examine some aspects of the volatile mode of action of these fungicides, and (c) determine factors effecting ascocarp formation in Sphaerotheca pannosa var. rosae. Abstracts of portions of this work have already been published (24,25).

TEXT CONTINUED FROM PAGE 6 TO PAGE 8.

MATERIALS AND METHODS

Greenhouse Control

The experimental fungicides nuarimol 9.46% EC (EL-228) [α-(2-chlorophenyl)-α-(4-fluorophenyl)-5-pyrimidinemethanol], bupirimate 26% EC (Nimrod[R]) (PP588) [5-butyl-2-(ethylamino)-6-methyl-4-pyrimidinyldimethylsulfamate], and fenapanil 24.2% EC (Sisthane[R]) (RH-2161) (α-butyl-α-phenyl-1$\underline{\text{H}}$-imidazole-1-propanenitrile) (Fig. 1), which are all reported to have volatile and systemic activity (34,38,49), were volatilized by heating in commercial greenhouses ranging in area from 595 m^2 to 1254 m^2 and in volume from 1886 m^3 to 4946 m^3 at three locations using the rose cultivar 'Forever Yours'. Applications were made at night with all vents closed and greenhouses were maintained at 15-18°C.

All fungicides were vaporized by applying the formulation to cold steam pipes then heating the pipes at 105°C for a specified time. In addition, fenapanil was divided equally between two 1.5 liter cans and heated at 150°C on calibrated hotplates in one experiment and two thermostatically controlled electric skillets heated at 175°C in another experiment, placed 1/3 the distance from each end of the greenhouse. Following treatment, greenhouses were vented before entry.

Disease severity was measured on 28 or 30 rapidly growing shoots per treatment and the mean for each treatment calculated. The number of powdery mildew colonies were counted on both surfaces

NUARIMOL   (EL-228)
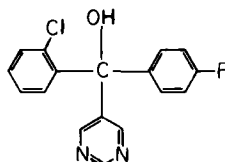
Chemical Name: α-(2-chlorophenyl)-α-(4-fluorophenyl)-
5-pyrimidinemethanol

Manufacturer: Eli Lilly and Company
Oral LD_{50} (female rat): 2065 mg/kg

FENAPANIL   (SISTHANE[R])   (RH-2161)

Chemical Name: α-butyl-α-phenyl-1H-imidizole-
1-propanenitrile

Manufacturer: Rohm and Haas Company
Oral LD_{50} (rat): 1590 mg/kg

BUPIRIMATE   (NIMROD[R])   (PP588)
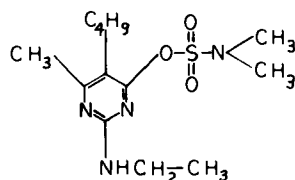
Chemical Name: 5-butyl-2-(ethylamino)-6-methyl-
4-pyrimidinyldimethylsulfamate

Manufacturer: ICI United States Inc.
Oral LD_{50} (female rat): 4000 mg/kg

Figure 1.   Structures of nuarimol, fenapanil and bupirimate.

of the first four or five unfolded five-leaflet leaves on each

shoot with the aid of a magnifying lamp.

Untreated control plots were not possible in the fumigated

greenhouses. An estimate of the efficacy of fumigation was made

by measuring disease severity before and after treatment. In

those cases where no disease severity measurements were taken

before treatment, greenhouses with uniform powdery mildew infection

were selected and fumigation treatments were compared with sprayed

treatments and unsprayed check plots in separate greenhouses.

Mode of Action

A. Growth Chamber Experiments. Preliminary fumigation experi-

ments were conducted on whole plants in Percival Controlled Environ-

ment growth chambers fitted with exhaust ducts to continually

exchange the air every 15 minutes. Fungicides were volatilized on

heated soldering irons that had been dipped into the emulsifiable

concentrates or on hotplates in shallow cast iron containers to

which copper-constantan thermocouples were attached and connected

to a chart recorder for temperature monitoring.

B. Fumigation Chamber Experiments. Subsequent experiments were

conducted in a specially designed fumigation chamber which could be

easily cleaned between experiments to remove fungicide residues and

was large enough to allow treatment of 4 rose plants in 6-inch pots

at one time (Fig. 2). It consisted of a 0.1 m^3 capacity bell jar

resting on a stainless steel base sealed with stopcock grease. Two
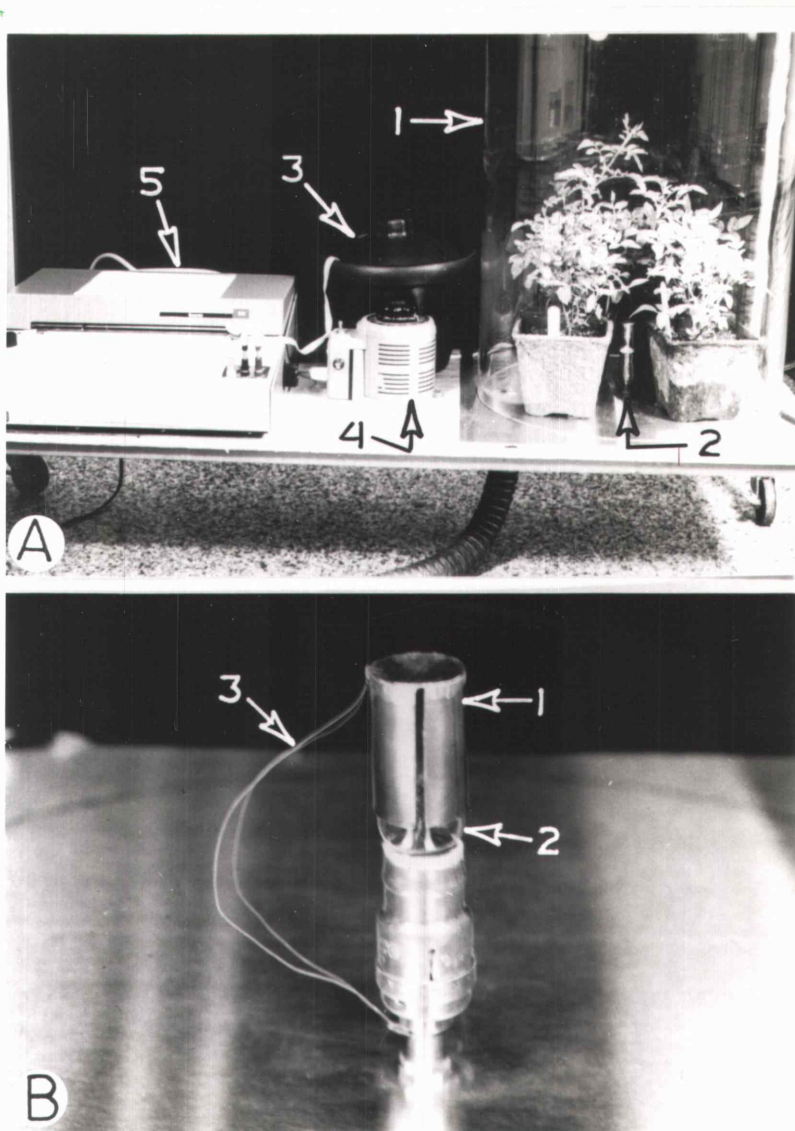
Figure 2. Fumigation chamber detail. <u>A</u>. (1) Bell jar on stainless steel base. (2) Fungicide receptacle and heat source. (3) Thermocouple ice bath. (4) Temperature control rheostat. (5) Chart recorder. <u>B</u>. (1) Copper jacket and fungicide receptacle. (2) 15-watt lamp. (3) Thermocouple.

holes in the base were plugged with rubber stoppers during fumigation and a hose fitted to an exhaust fan was connected to evacuate the chamber for 30 minutes after each treatment. The heat source was a rheostat controlled 15-watt lamp covered with a copper jacket which had a depression on top to serve as a fungicide receptacle. A copper-constantan thermocouple was attached to the receptacle and connected to a chart recorder for temperature measurement.

Disease-free leaf tissue was collected from plants maintained in individual filtered air chambers (Fig. 3) which were a modification of those developed by Coyier (12). Five-inch round plastic pots were fitted flush under the lip at the top with an 8-inch donut shaped ring made of 3/4-inch marine plywood which was sealed and secured to the pot with caulking. Rooted cuttings were stripped of leaves, rinsed clean in water, dipped in 95% ETOH, immersed in 0.75% sodium hypochlorite for 10 min and potted in a mix of 1:1:1:1 by volume of sterile soil, peat moss, sand and vermiculite. The sealed end of a clear polyethylene bag was cut off to form a straight tube. One end of the bag was secured around the perimeter of the plywood ring with a large plastic electrical tie. The top of the bag was gathered and secured with a twist tie around a cork with a hole drilled longitudinally through its center and plugged lossely with cotton to allow air to escape. Filtered air entered the chamber through a quick-disconnect fitting in the plywood ring. Supplemental watering and liquid fertilizer were supplied periodically to each plant via a small plastic tube entering the chamber through the
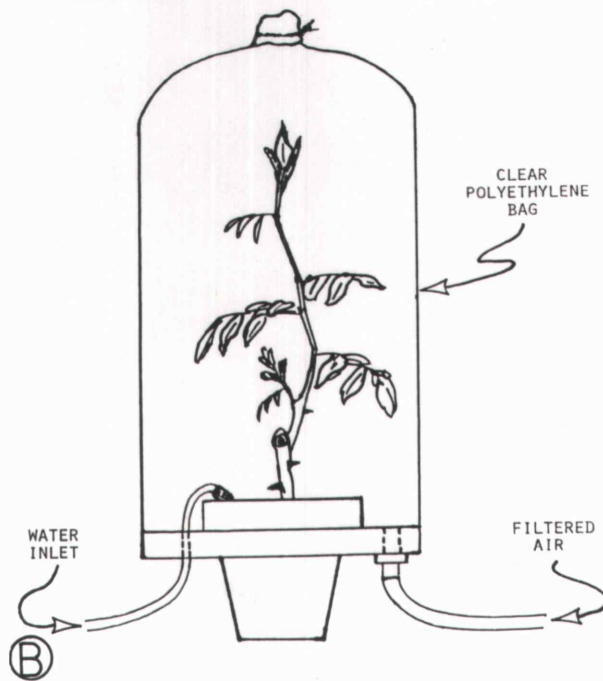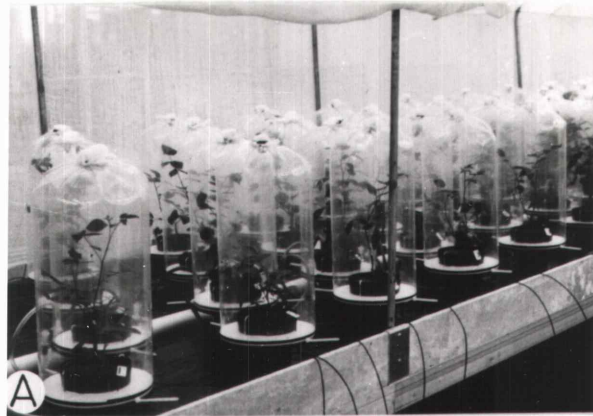
Figure 3. Filtered air chambers for maintaining powdery mildew
cultures and disease-free leaf tissue. A. Arrangement
of chambers in the greenhouse. B. Chamber detail.

ring.   Routine watering was accomplished by placing pots on a
fibrous mat which was saturated daily.

In experiments where conidia were fumigated in situ then
transferred to disease-free leaf tissue, old conidia were blown
off source plants naturally infected in the greenhouse 36 hr
before treatment using an empty portable pesticide mist blower.
In experiments where conidia were transferred to disease-free
leaf tissue before treatment, old conidia were removed from source
plants 24 hr before treatment in the same manner.   Detached leaf-
lets or leaf sections were floated on distilled water in glass or
ceramic spotplates and inoculated by touching a small sable hair
artist's brush to an actively sporulating colony and gently drawing
the brush across a fine nylon screen suspended over the leaf tissue.
Leaf tissue was fumigated in the spotplates immediately after inocu-
lation in some experiments.   When 24-hr-old colonies were fumigated,
conidia were first germinated for 4 hr in moist chambers containing
10% glycerol (w/w) solution to maintain relative humidity (RH) at
about 97%, then held for 20 hr in moist chambers containing a 52%
glycerol solution to maintain about an 80% RH (50).   Conidia sown
to leaf tissue after treatment were then germinated and maintained
in the same manner.

Microscopic examination was accomplished using a Zeiss compound
microscope equipped with vertical illumination and Epiplan HD 8/0.2
and 16/0.35 objectives.   Colony growth and fungicide effect were
rated by observing all colonies on each leaf section and estimating

TABLE 1. Colony growth ratings and fungicide effect ratings.

Rating	Description

Colony Growth Rating System:

0	No germination.
1	Germination - length of germ tube at least 1/2 the length of the conidium, but no elongating secondary hyphae (ESH) >12 microns beyond the first appressorium.
2	ESH >12 microns beyond the first appressorium, but no branching.
3	Branched hyphae.
4	Developing conidiophores.
5	Sparse sporulation.
6	Normal sporulation.

Fungicide Effect Rating System:

0	No fungicide effect.
1	Very slight effect. Swellings (protuberances) not noticeable and difficult to find. Small amount of twisted and/or aerial hyphae with abnormal appressoria.
2	Slight effect. Swellings easy to find but occur on less than 25% of active hyphal tips. Colonies continue with little apparent slowing of growth. Twisted and aerial hyphae obvious.
3	Moderate effect. Many swellings. More than half of the hyphal tips with swellings continue growth beyond swellings or by branching behind swellings.
4	Severe effect. Many swellings. Less than half of the hyphae with swellings continue to grow.
5	Colonies dead. Most or all hyphal tips swollen.

the average for that sample using the systems listed in Table 1.

Leaf discs with young, actively growing powdery mildew colonies

were placed in the fumigation chamber after each treatment for

a minimum of 12 hr as a check for volatile residues.

Specimens for scanning electron microscopy were fixed in a

5% gluteraldehyde - 0.1 M phosphate buffer solution, dehydrated

through 50%, 70% and 95% ETOH and through 50%, 70% and 100% tri-

chlorotrifluoroethane, dried in a Bomar SPC-900 Critical Point

Dryer, mounted with colloidal silver paint and coated with 60-40%

gold-palladium in a VE-10 Varin Vacuum Evaporator.

Ascocarp Induction

Indole-3-acetic acid (IAA), Indole-3-butryic acid (IBA), and

gibberellic acid (GA) lanolin pastes were made by first dissolving

each hormone in 95% ethanol, diluting with distilled water to bring

the ethanol concentration to 23.75%, then serially diluting with

stock 23.75% ethanol to form 10^{-2}, 10^{-3} and 10^{-4} M solutions of

each hormone. Kinetin was first dissolved in 0.1 N NaOH, diluted

2X with distilled water to make a 10^{-2} M solution, then serially

with distilled water to make 10^{-3} M and 10^{-4} M solutions. Anhydrous

lanolin (4.5 g) was mixed with 0.5 ml of each solution to form 10^{-3},

10^{-4} and 10^{-5} M pastes. Controls were a lanolin-ethanol paste, a

lanolin-NaOH paste, lanolin only and untreated. In all experiments

in which plants were treated with plant growth hormones, flower parts

were excised using a razor blade to make a cut at the base of the

sepals and perpendicular to the vertical axis of the flower. The
lanolin-hormone pastes were then applied to the cut surfaces.
Rose cv. Dwarf Crimson Rambler was used in all experiments involving
ascocarp induction.

All 16 treatments were applied to each of nine plants chosen
for uniformity of flower and disease development. Buds were closed
and hypanthia were completely infected with powdery mildew. Plants
were maintained in the greenhouse under 15 hr daylength and a temper-
ature range of 17°C to 27°C and examined daily for three mo. An
additional plant which had 1/2 opened buds and tan colored pannose
mycelium on the hypanthia was treated with 10^{-3} M, 10^{-4} M and 10^{-5}
M IBA pastes. Six randomly selected flowers were treated with each
hormone concentration, lanolin + ETOH and lanolin only, and six
left untreated. The plants were maintained in the greenhouse as
in the previous experiments.

The effect of predisposing plants by low temperature during
disease development and subsequent treatment with IBA was tested.
Three plants were maintained in a growth chamber for three mo with
12 hr daylength and night and day temperatures of 11°C and 21°C,
respectively. Three greenhouse-grown plants were maintained for
three mo with 15 hr daylength and night and day temperatures of
17°C and 27°C, respectively. Following predisposition treatments
three randomly selected flowers on each plant were treated with
10^{-4} M IBA, 10^{-4} M IAA, lanolin + ETOH, lanolin only and a minimum
of three flowers left untreated. All six plants were subsequently

maintained in the growth chamber as described and observed daily for three mo.

In experiments testing the effects of fertility levels and daylength on ascocarp production, plants were either not fertilized or fertilized weekly with Hoagland's solution (32) in the greenhouse or shadehouse. Supplemental lighting (15 hr daylength) was maintained in the greenhouse with General Electric high intensity sodium discharge lamps. Night and day temperatures during 12 hr periods were 17°C and 27°C, respectively. Short daylength (11 hr) was maintained in the greenhouse by covering plants with a black plastic dropcloth.

Detached rose leaf sections for support of powdery mildew cultures were maintained using a system developed by Coyier (14). Four leaf sections were fastened to a glass microscope slide using tape with a hole for each section. A thin strip of filter paper sandwiched between the leaf tissue and the slide served as a wick to provide water to the tissue.

Single-spore isolates were all obtained from colonies in close proximity to developed cleistothecia and transfers were accomplished using a human eyelash as a probe which was glued to the end of a 1 ml plastic disposable pipette (13). Powdery mildew conidia were observed using a stero microscope and the desired conidia singly transferred to the detached leaf tissue then examined for damage and orientation under a Zeiss compound microscope equipped with vertical illumination. Several conidia were seeded per disc and

all but one colony was removed 24 hr after inoculation. Conidia from sporulating colonies on detached leaf tissue were mass transferred to the youngest fully expanded leaves on an individual disease-free rose plant maintained in isolation chambers as described for the fumigation experiments (Fig. 3). Inoculated leaves were marked at the base of the midrib with india ink and observed daily for colony growth. Single-spore isolates were combined by mass inoculating individual leaflets on a disease-free rose plant, each with a different fungal isolate.

RESULTS

Greenhouse Control

A comparison of the efficacy of bupirimate and nuarimol applied by spraying and by fumigation is given in Table 2. Treatments were applied in separate greenhouses of equal size (1254 m^2; 4946 m^3). Bupirimate spray treatment resulted in excellent control of powdery mildew but was completely ineffective as a fumigant, while nuarimol completely controlled the disease by either spray or fumigant application.

Particularly striking was the improved general appearance of the plants. Although the disease control for nuarimol was the same in both treatments, plants from the fumigated house were of better quality than those from the sprayed house due to the lack of spray deposit and water marks on the foliage and blossoms.

Because the disease reappeared within two wks after the final treatment in all of the greenhouses except the one treated by nuarimol fumigation, it became necessary to spray with triforine, a standard for rose powdery mildew control. Three sprays were applied in each of the three greenhouses at weekly intervals and the disease severity was determined one wk after the final spray treatment (Table 3). Triforine provided fair control in the house which had previously been fumigated with bupirimate, but disease increased in the other two greenhouses. The greenhouse which had no further treatment after having previously been fumigated with

TABLE 2. Greenhouse control of <u>Sphaerotheca</u> <u>pannosa</u> on 'Forever Yours' roses by
volatilized compared with sprayed fungicides at Vashon, WA.

Fungicide[a]	Application Method	Rate (kg a.i./ha)	Colonies/Shoot[c] Initial	Colonies/Shoot[c] Final	% Disease[d] Control
Bupirimate 26% EC	spray	0.91	43.20	1.67	96
Bupirimate 26% EC	fumigation[b]	0.91 (23.8) (mg/m^3)	126.73	301.93	0
Nuarimol 9.46% EC	spray	0.34	72.40	0.0	100
Nuarimol	fumigation[b]	0.34 (8.6) (mg/m^3)	122.20	0.0	100

a) Each greenhouse was treated four times, at 7-day intervals.
b) Formulation applied to steam pipes and heated to 105°C for 4-6 hr.
c) The number of fungal colonies on the first five unfolded 5-leaflet leaves on
30 actively growing shoots per greenhouse were counted immediately before the
initial treatment and one wk after the final.
d) % disease control = $\frac{\text{(initial colony count)} - \text{(final colony count)}}{\text{inital colony count}}$ X 100.

TABLE 3. Duration of powdery mildew control in the greenhouse on 'Forever Yours' roses five wk after final fumigation with nuarimol at Vashon, WA.

Previous Treatment[a]	Treatment[b]	Rate (kg a.i./ha)	Colonies/Shoot[c] Inital	Final
Bupirimate spray	Triforine spray 18.2% EC	0.56	1.67	17.13
Bupirimate fumigation	Triforine spray 18.2% EC	0.56	301.93	15.27
Nuariamol spray	Triforine spray 18.2% EC	0.56	0.0	1.57
Nuarimol fumigation	None	---	0.0	0.07

a) See Table 2 for details of previous treatments.
b) Three sprays applied once per wk beginning two wk after previous treatment.
c) The number of fungal colonies on the first five unfolded 5-leaflet leaves on 30 actively growing shoots per greenhouse were counted 1 wk after the last treatment. Initial counts were the same as final counts in the previous treatments.

nuarimol showed a small increase in disease incidence, still well below acceptable levels. The mean of 0.07 fungal colonies per shoot represents two colonies in the entire sample. No further disease ratings were made in this greenhouse, but without any further fungicide treatment disease incidence did not become high enough to warrant treatment for six mo, while the other three greenhouses required continual standard treatment. The disease increased slowly in severity over this time period in contrast with the rapid increase normally observed.

Two heating times were tested with nuarimol in a commercial greenhouse (929 m^2; 3445 m^3) at Forest Grove, OR (Table 4). Eighty-two percent control was achieved with short duration nuarimol fumigation, but the number of powdery mildew colonies per shoot was unacceptably high based on industry quality standards. Three to four hour fumigation time increased disease control to 99% (0.23 fungal colonies/shoot), representing seven colonies in the entire sample. Microscopic examination revealed that four of those seven colonies were dead.

In subsequent tests at Vashon, WA, nuarimol provided equally good control by heating the fungicide in 1.5 liter cans on calibrated hotplates or by heating the fungicide on steam pipes. This suggested that it is unnecessary to distribute the fungicide over a wide surface area in order to achieve uniform vapor distribution throughout the greenhouse.

The volatile activity of fenapanil was tested at Hillsboro, OR by comparing the disease incidence in a fumigated greenhouse with

TABLE 4. The effect of exposure time on the efficacy of nuarimol
for control of powdery mildew on 'Forever Yours' roses
at Forest Grove, OR.[a]

Duration of Heat Application	Colonies/Shoot[b]		% Disease Control[c]
	Initial	Final	
1 1/2 - 2 hr	188.13	33.50	82
3 - 4 hr	33.50	0.23	99

a) Nuarimol (9.46% EC) formulation applied to steam pipes (8.7
mg a.i./m^3, 0.33 kg/ha) and heated to 105°C once per wk for
four wk.
b) Each mean represents the number of fungal colonies on the
first four unfolded 5-leaflet leaves on 30 actively growing
shoots taken at random. Final counts taken one week after the
last fumigation.
c) % disease control = $\dfrac{\text{(initial colony count)} - \text{(final colony count)}}{\text{initial colony count}}$ X 100.

that in another sprayed with the same fungicide (Table 5). Both
greenhouses were of equal size (595 m^2; 1886 m^3). Although no
pretreatment disease ratings were made, the same low incidence
of disease was observed in both greenhouses at the beginning of
the test.

At Vashon, WA two greenhouses (1254 m^2; 4946 m^3) were chosen
with apparent uniform disease incidence. Fenapanil was volatilized
in one greenhouse and compared with sprayed plots and unsprayed
check plots in the other greenhouse (Table 6). The fungicide was
placed in two 1.5 liter cans on calibrated hotplates to determine
whether the vapors would distribute sufficiently from point sources
to provide adequate control. Because all the liquid was not driven
off in three hr of heating, the chemical was reheated for three hr
each night, and new fungicide added at 7-day intervals.

Fair control was obtained with the spray treatment but the
colony count was above commercially acceptable levels. Compared
with spray and check treatments, the volatilized fungicide gave
excellent disease control (Table 6). Plants in the fumigation
treatment lacked spray residue and water marks. The foliage was
more dense, had higher lustre, and deeper green color. The general
appearance of fumigated plants was far superior to spray treatment.
Although the rate of active ingredient in the fumigation treatment
was more than double that used in the previous treatment (Table 5),
no phytotoxicity was observed.

Comparisons of the efficacy of triforine and fenapanil spray
treatments with volatilized fenapanil at Forest Grove, Oregon are

TABLE 5. Control of powdery mildew on 'Forever Yours' roses by volatilization and spray treatment of fenapanil at Hillsboro, OR.

Treatment[a]	Rate (kg a.i./ha)	Colonies/Shoot[c] Final Count
Fenapanil spray	0.95	0.0
Untreated	---	2.14
Fenapanil fumigation[b]	0.48 (15.1) (mg/m^3)	0.14

a) Treatments conducted in two greenhouses four times on a 14-day schedule. Triton AG-98 at 0.3 ml/1 used as a surfactant in spray treatment.
b) 118 ml of formulation was applied to steam pipes and heated to 105°C for four hr.
c) The mean number of fungal colonies on the first four unfolded 5-leaflet leaves on seven actively growing shoots per each of four replications were counted one wk after the final treatment.

TABLE 6. Control of powdery mildew on 'Forever Yours' roses
by fenapanil volatilized and sprayed in greenhouses
at Vashon, WA.

Treatment[a]	Rate (kg a.i./ha)	Colonies/Shoot[c] Final Count
Fenapanil spray	0.91	33.68
Untreated	---	293.64
Fenapanil fumigation[b]	1.36 (34.4) (mg/m^3)	6.61

a) Treatments conducted in two greenhouses four times on a
7-day schedule. Triton AG-98 (0.3 ml/l) used as a sur-
factant in spray treatment.
b) 710 ml of formulation was divided between two 1.5 liter
cans on hotplates placed 1/3 the distance from each end
of the greenhouse at 150°C for three hr each 24 hr. The
chemical was replenished every seven days.
c) The mean number of fungal colonies on the first four
unfolded 5-leaflet leaves on seven actively growing
shoots per each of four replications were counted one
wk after the final treatment.

summarized in Table 7. The respective greenhouse sizes for the three treatments were 743 m^2 (2568 m^3), 929 m^2 (3445 m^3) and 613 m^2 (2009 m^3). Excellent control was achieved by both fenapanil treatments but their efficacy cannot be accurately compared because the initial counts differed among the houses. The general appearance of plants in the fumigated greenhouse was superior to those in the sprayed greenhouse when considering the density, color and lustre of the foliage and blossoms and the lack of spray residue.

Triforine, one of the standard fungicides used in the cut rose industry, was totally unsatisfactory. Although the colony counts per shoot did not increase as much as the controls, efficacy was unacceptable. It was surprising that acceptable control was obtained with triforine at Vashon, WA (Table 3) while applying a higher rate at Forest Grove, OR resulted in poor control (Table 7).

Mode of Action

A. Preliminary Experiments in Growth Chambers. Two rose plants, cv. Dwarf Crimson Rambler, uniformly infected with rose powdery mildew were fumigated with nuarimol (approx. 75 mg a.i./m^3) or bupirimate (125 mg a.i./m^3) in growth chambers for two hr once per wk for two wk using soldering irons as a heat source. Two check plants in a third chamber were not treated. Plants were rated for percent infection one wk after the final treatment. Infection was 13% in the nuarimol treatment compared with 35% in the bupirimate treatment (Table 8). Because temperature control and precise measurement of

TABLE 7. Control of powdery mildew on 'Forever Yours' roses by volatilized and sprayed fungicides in greenhouses at Forest Grove, OR.

Treatment[a]	Rate (kg a.i./ha)	Colonies/Shoot[c] Initial	Colonies/Shoot[c] Final	% Disease Control[c]
Triforine spray	0.90	14.07	106.60	0
Untreated	---	18.00	391.89	-
Fenapanil spray	0.98	51.11	4.07	92
Untreated	---	22.25	181.36	-
Fenapanil fumigation[b]	0.46 (14.1) (mg/m^3)	13.82	2.07	85

a) Treatments applied four times at weekly intervals. Triton AG-98 (0.3 ml/1) used as a surfactant in both spray treatments. Greenhouse size: area = 613 m^2; volume = 2009 m^3.

b) 118 ml of formulation was divided between two electric skillets placed 1/3 the distance from each end of the greenhouse and heated at 175°C for four hr each 24 hr. The chemical was replenished every seven days.

c) The mean number of fungal colonies on the first four unfolded 5-leaflet leaves on seven actively growing shoots per each of four replications were counted immediately before the initial and one wk after the final treatment.

d) % disease control = $\frac{\text{(initial colony count)} - \text{(final colony count)}}{\text{initial colony count}}$ X 100.

TABLE 8. Effect of heating fungicides on control of rose
powdery mildew in controlled environment chambers.

Fungicide[a]	Rate (mg a.i./m^3)	% Infection Final Rating
Nuarimol	74.4	13
Bupirimate	125.2	35
Untreated	0	85

a) Fungicides were volatilized for two hr once per wk for
two wk. Two plants per treatment were rated one wk
after final treatment by estimating the percent infec-
tion on the new growth of each plant.

the pesticide were not possible, soldering irons were not used again.

Six plants per treatment were fumigated with nuarimol and bupirimate once per wk for four wk. Fungicides were heated to 105°C and 185°C for 4.5 hr and disease ratings were made one wk after the final treatment. Nuarimol completely controlled powdery mildew at both temperatures, while bupirimate was less effective when volatilized at 105°C than at 185°C (Table 9).

The volatility of triforine and fenapanil was tested using whole plants and by exposing infected leaf discs only. After three weekly treatments all treated plants were disease-free while plants in the untreated control were completely infected. However, infected leaf discs which were removed and examined after each fumigation were unaffected by the fungicides, suggesting that either 1) several treatments were required before the fungicides were effective or 2) that the fungicides from this and previous experiments were sorbed by materials in the growth chambers and subsequently released as vapors even though the inside of the chambers had been thoroughly washed with a strong detergent solution between each experiment.

The second hypothesis proved correct. Six rose plants (cv. Dwarf Crimson Rambler), slightly infected with powdery mildew were placed in each of the three growth chambers in which fumigation experiments had been previously conducted. Five and seven days later powdery mildew conidia from heavily infected plants were

TABLE 9. The effect of volatile fungicides heated to 105°C and 185°C on rose powdery mildew in controlled environment chambers.[a]

Fungicide	Rate[b] (mg a.i./m^3)	Initial % Infection		Final % Infection	
		105°C	185°C	105°C	185°C
Nuarimol	72.2	100	100	0	0
Bupirimate	192.4	100	100	27	0
Untreated	0	100	100	98	100

a) Six plants per treatment were fumigated for 4.5 hr once per wk for four wk. Disease ratings were made immediately before initial and one wk after final treatment by estimating the percent infection on the new growth of each plant.
b) 0.5 ml of each formulation was used.

uniformly blown onto each plant. On day 15, all plants were
rated for percent disease (Table 10). Plants in the chamber
in which nuarimol had previously been volatilized (chamber NU)
were disease-free. Plants in the chamber in which bupirimate
and fenapanil had previously been volatilized (chamber BU-FE)
were 4% infected with powdery mildew, but the disease was restricted
to colonies existing on the older leaves before treatment. Colonies
were compact and abnormally raised with sharply defined margins.
Conidiophores were indistinguishable as separate structures and
the mycelium was aerial and abnormally twisted with conidial masses
in the center. Plants in the chamber not previously fumigated
(control) were completely infected with normal appearing powdery
mildew colonies (Fig. 4).

In order to remove the vapor phase effect of the fungicides
the following steps were taken; (a) disassembling and washing
chamber parts in detergent and 95% ETOH, (b) removal of all paint
inside chambers and refinishing and (c) replacement of most plastic
parts inside each chamber. Following these procedures the highest
infection achieved was 20.8% in chamber NU and 70.8% in chamber
BU-FE while untreated plants were completely infected. Although
no further attempts were made to remove the residual fungicide,
there were detectable levels of fungicidal activity in both growth
chambers six mo after the last fumigation experiments were concluded
and 12 mo after the last treatment of chamber NU. Growth chamber
fumigation experiments were abandoned.

TABLE 10. Residual activity in growth chambers of fungicides previously volatilized for control of rose powdery mildew.[a]

Growth Chamber[b]	% Infection
NU	0
BU-FE	4
Control	100

a) Six slightly infected rose plants (cv. Dwarf Crimson Rambler) were placed in each growth chamber at the beginning of the experiment. Plants inoculated at five and seven days. Disease ratings taken 15 days after beginning experiment by counting the number of leaves infected on each plant.
b) Nuarimol previously volatilized in growth chamber NU and both bupirimate and fenapanil previously volatilized in growth chamber BU-FE.
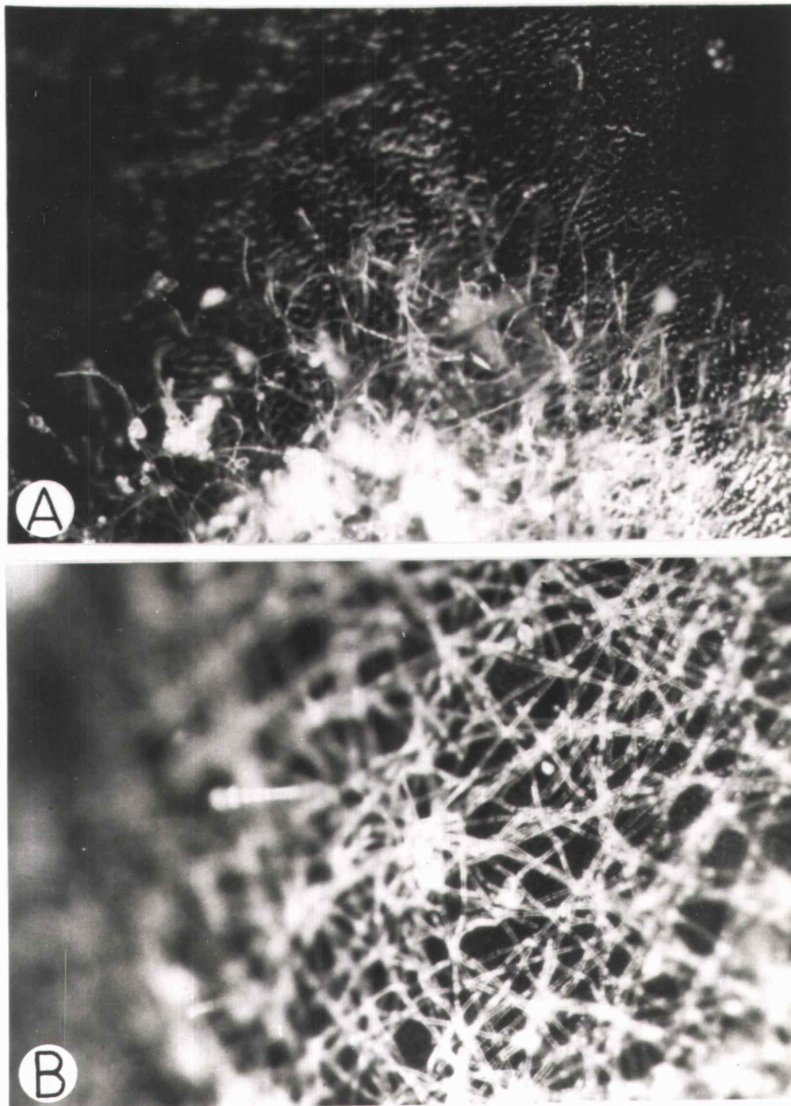
Figure 4. Residual fungicide activity in growth chambers. A. Effect
           of residual volatile activity from growth chamber pre-
           viously fumigated with bupirimate and fenapanil (chamber
           BU-FE) (X80). B. Normal colony development on leaf of a
           plant grown in an untreated growth chamber (X80).

B. Fumigation Chamber Experiments. Whole plants were fumigated to determine (a) the level of powdery mildew control when plants are exposed to the fungicide only during the fumigation period and (b) whether improved control would result from treatment intervals less than seven days. Four severely infected rose plants (cv. Dwarf Crimson Rambler) were treated four times for four hr at 4- and 7-day intervals with nuarimol (10 mg a.i./m^3) volatilized at 150°C and rated for disease incidence one wk after the final treatment. The experiment was repeated once. New growth on plants in the 4-day treatment (Table 11) were disease-free and most colonies on the old growth appeared dead. Two weeks after the final treatment slight abnormal development was observed in several colonies, but no conidiophores or conidia developed.

Plants in the 7-day treatment also showed no disease development on any of the new growth (Table 11). Foliage had an excellent appearance with no phytotoxicity. However, colonies already established at the time of the first fumigation continued abnormal development. Microscopically, mycelium had numerous short branches with swellings and constrictions giving the colonies a dense, lumpy appearance compared to normal colonies (Fig. 5). Although about 50% of each colony examined were turgid and appeared alive, the only conidia present were dessicated and appeared to have been produced before fumigation. All young hyphal strands near the colony margins had branched normally but were severely dessicated with swellings on the tips.

TABLE 11. Comparative control of rose powdery mildew on whole plants fumigated
at 4- and 7-day intervals.[a]

| Treatment Interval | Infection[b] | | % Disease Control[c] |
	Initial	Final	
4-day	100	1	99
7-day	100	15	85

a) Plants were maintained in growth chambers and transferred to a fumigation
chamber for treatment. Plants were fumigated with nuarimol (10 mg a.i./m^3)
four times for four hr per treatment.
b) Values are means of ratings on four plants, repeated twice.
c) % disease control = $\dfrac{\text{(initial infection)} - \text{(final infection)}}{\text{initial infection}}$ X 100.
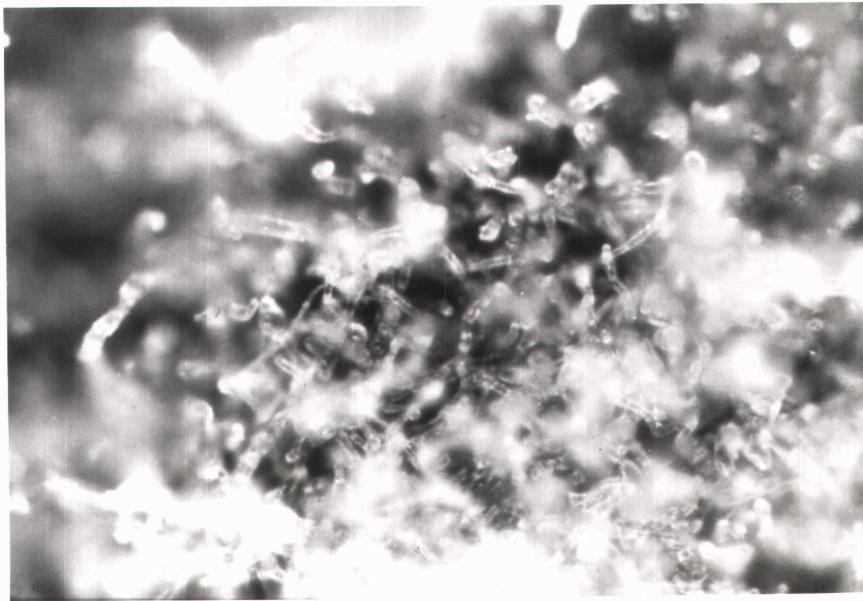
Figure 5. Mature colony with abnormally numerous branches and swellings resulting from fumigation with nuarimol (X160).

Conidia were sparse in some colonies two wk after the final fumigation in the 7-day treatment and were mass transferred to 12 untreated detached leaf sections. Germination and fungicide effect were examined 48 hr later. Two leaf sections each had one germinated conidium. The mycelium on one leaf section with a colony growth rating of 2.0 touched the leaf surface in only a few places. The mycelium in the second colony (rated 3.0) was slightly distorted with typical nuarimol-caused swellings. A third leaf section had 58 developing colonies with no visible effect from nuarimol (rated 3.1). Conidia on this disc had been transferred from petals of a flower which opened after the last fumigation, indicating that hyphae may have been protected in the unopened bud much the same as overwintering powdery mildew mycelium is protected in dormant vegetative buds. At 72 hr after inoculation the colony ratings were 3.0, 3.4 and 4.0, respectively. The effects of nuarimol on the first two colonies were obviously diminishing.

Actively growing one-day-old powdery mildew colonies on detached leaf sections had ceased growth 24 hr after a two hr exposure to heated nuarimol at a rate of 1.8 mg a.i./m^3 or two, four or six hr exposure to 10 mg a.i./m^3. Colony development was correlated with fungicide dose (Table 12). Swellings or protuberances typical of nuarimol treatment were present on most hyphal tips in all treatments (Figs. 6 and 7). Hyphal tip swellings were still evident and all established colonies were severely dessicated 72 hr after treatment. In contrast, conidia with germ tubes that had not established

TABLE 12. The effect of nuarimol fumigation on 24-hr-old colonies of rose powdery mildew.[a]

Nuarimol Rate	Exposure Time (hr)	Colony Growth Rating[b] 24 hr	48 hr	Growth % of 48 hr Control
1.8 mg a.i./m^3	2	3.29[c]	3.29	73.95
Untreated Control		3.59	4.45	
10 mg a.i./m^3	2	2.45	2.45	62.09
Untreated Control		3.43	3.96	
10 mg a.i./m^3	4	2.58	2.58	66.78
Untreated Control		3.36	3.86	
10 mg a.i./m^3	6	2.41	2.41	56.64
Untreated Control		3.35	4.11	

a) Actively growing one-day-old colonies were exposed to 1.8 mg a.i./m^3 nuarimol heated to 105°C for two hr or 10 mg a.i./m^3 for two, four or six hours.

b) Colony growth rating: 0 = no germination; 6 = normal sporulation. Rated 24 and 48 hr after treatment.

c) Each treatment and its corresponding untreated control are paired samples. Ratings are means of 400 colony observations: 25 observations per each of four replications repeated four times. All treatments were significantly different from corresponding untreated control (p = .05).
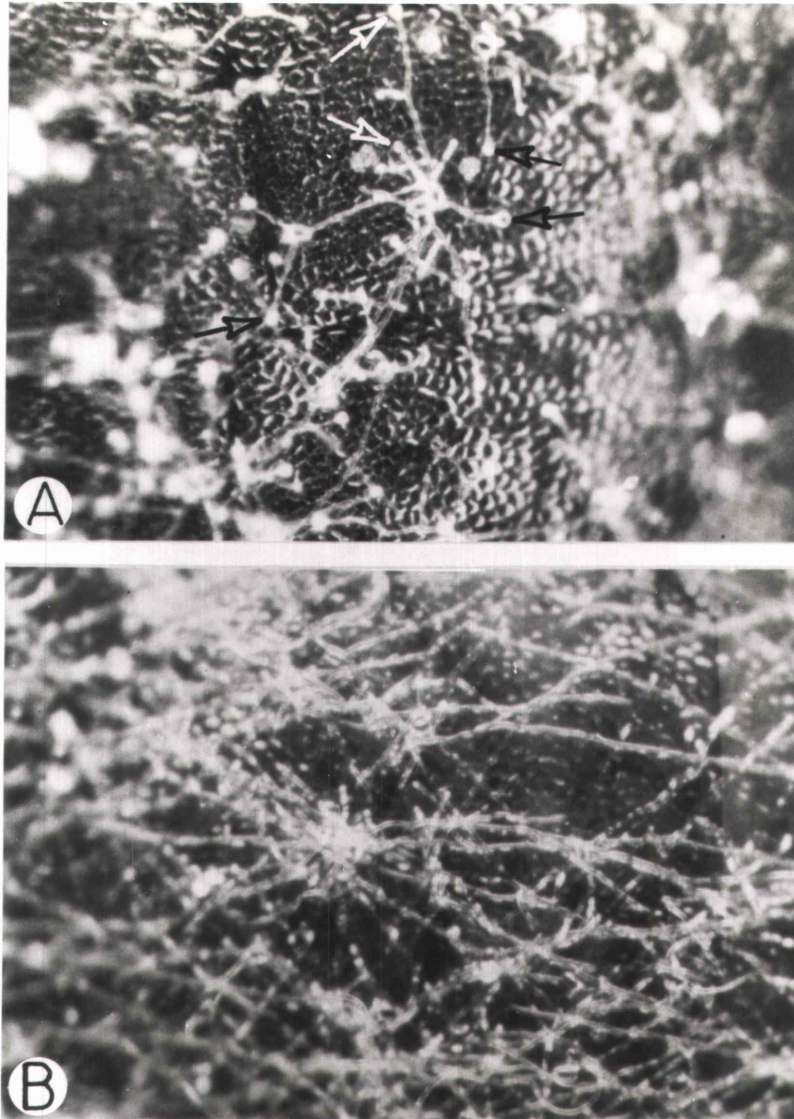
Figure 6. The effect of volatilized nuarimol on rose powdery
mildew colony development. A. One-day-old powdery
mildew colonies 24 hr after exposure to 10 mg a.i./m^3
nuarimol showing typical hyphal tip swellings (arrows)
(X80). B. Normal colony in the untreated check (X80).
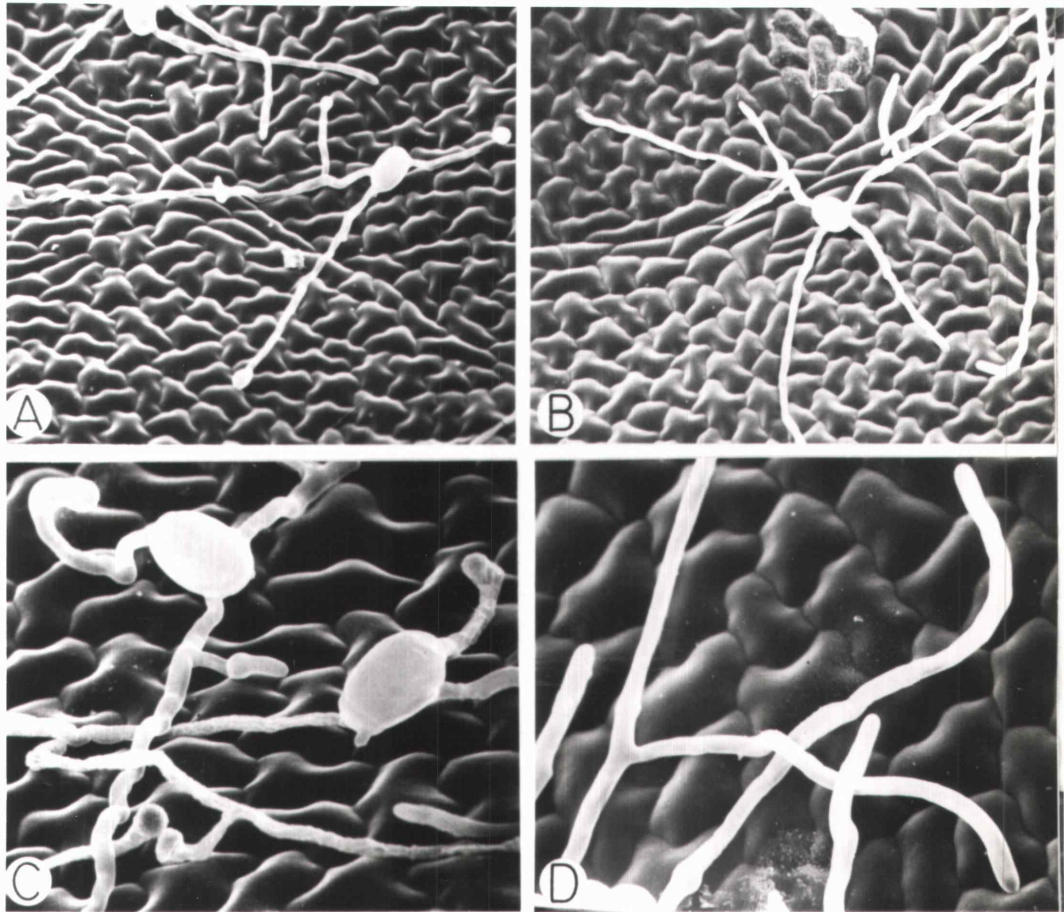
Figure 7. Scanning electron micrographs of one-day-old colonies after exposure to 10 mg a.i./m^3 nuarimol. A and C treated; B and D untreated. A and B X400; C and D X1000). (Electron microscopy by Al Soeldner.)

compatible relationships with the host and had ceased hyphal growth before fumigation remained turgid (Fig. 8).

Formulated fungicide was used in all tests. The vapor pressure of technical nuarimol is 2×10^{-8} Torr, but according to the manufacturer volatilization occurs at ambient temperatures when the fungicide is dissolved in certain solvent systems. Young powdery mildew cultures were not affected by four hr exposure to the unheated fungicide but all cultures were killed when the fungicide was heated to 150°C (Table 13). There was no morphological change in the fungus after heat treatment of the solvent blank for six hr but there was a slight decrease in fungal development as indicated by both the 48- and 72-hr colony growth ratings.

Because the materials contacted by the vaporized fungicide inside the fumigation chamber were all non-porous, it was expected that any buildup of residue could be easily removed. Young powdery mildew colonies on leaf sections were exposed in the chamber for 6 and 12 hr after the first 4-hr experiment, after ten 4-hr volatilizations representing 40 hr of treatment, and again after all inside chamber surfaces had been washed with dimethyl sulfoxide (DMSO) followed by 95% ETOH. No fungicide effect was detected following exposure of the fungus for 12 hr in the 4-hr chamber but there was a moderate effect on the fungus incubated in the 40-hr chamber (Table 14). No colonies were killed but more than half the hyphal tips were swollen. After the solvent wash there was no residual effect of nuarimol detected at ambient temperature or when the fungicide receptacle was heated to 150°C for six hours.
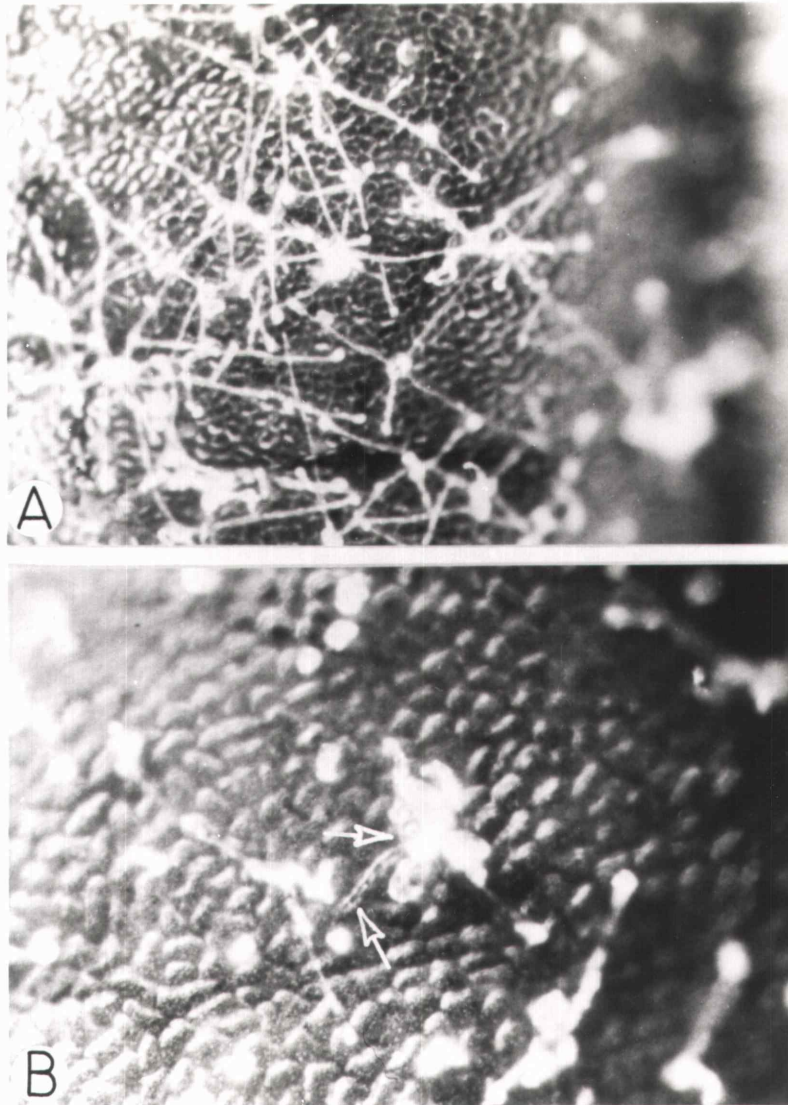
Figure 8. Nuarimol effect on compatible and incompatible powdery
           mildew colonies. A. One-day-old colonies fumigated
           with nuarimol showing severely dessicated hyphae 72
           hr after treatment (X80). B. Germ tube and conidium
           (arrows) which have not established compatible relation-
           ship with host appear turgid 72 hr after treatment (X160).

TABLE 13. The effect of heated and unheated nuarimol and the heated solvent blank on rose powdery mildew in a fumigation chamber.

Treatment	Fungicide Effect[a]		Colony Growth[b]	
	24 hr	48 hr	48 hr	72 hr
Nuarimol heated[c]	5.0[e]	5.0	2.5	2.5
Untreated Control	0	0	3.5	5.4
Nuarimol unheated	0	0	3.5	5.2
Untreated Control	0	0	3.6	5.3
Solvent heated[d]	0	0	4.3	5.1
Untreated Control	0	0	4.7	5.4

a) Fungicide effect rating: 0 = no visible effect; 5 = colony dead.
b) Colony growth rating: 0 = no germination; 6 = normal sporulation.
c) 2 µl (1.8 mg a.i./m^3) of nuarimol heated at 150°C or unheated for four hr.
d) 10 ml of the solvent blank heated at 150°C for six hr.
e) Colonies were 24 hr old at treatment. Values are means of 16 observations per treatment. Treatments and corresponding checks are paired observations.

TABLE 14. The effect of residual nuarimol on rose powdery mildew in a fumigation[a] chamber after 4 and 40 hr of volatilization and after solvent washing.

	Fungicide Effect[b]			
Colony Exposure (hr)	No. of 4 hr treatments with 10 mg a.i./m^3		After Solvent Wash	
	1	10	Without Heat	With Heat
0	0[c]	0	0	0
6	0	3.4	0	0
12	0	3.8	0	--

a) 10 mg a.i./m^3 of nuarimol was volatilized during each increment of four hr to a total of 40 hr. After testing the residual fungicide effect, all inside surfaces washed with DMSO and 95% ETOH and again tested without heat and with heated fungicide receptacle at 150°C.
b) Fungicide effect ratings: 0 = no effect; 5 = colonies dead.
c) Values are means of 200 colony observations: 25 observations per leaf disc, eight leaf discs per treatment.

A controlled determination of fungicide sorption by fiberglass insulation, 4 mil polyethylene sheeting and greenhouse fiberglass sheeting showed that there was moderate sorption of nuarimol on fiberglass insulation as indicated by a fungicide effect rating of 2.7 (Table 15). However, there was very little observed sorption by the polyethylene sheeting and none by the fiberglass sheeting.

Germination of rose powdery mildew conidia was not impaired when whole plants were fumigated with nuarimol and conidia were transferred to untreated leaf tissue. Developing colonies showed typical fungicide effects. Germination of conidia in controlled experiments was not effected by fumigation at the same rates and length of exposure that killed young colonies (Table 16). Only at a high dosage (45 mg a.i./m^3 for four hr) was germination significantly reduced.

In order to determine the dosage necessary to arrest developing hyphae from conidia, conidia were fumigated at 1.8 mg a.i./m^3 for two hours or 10 mg a.i./m^3 for two, four or six hours, then transferred to untreated leaf sections. Results are given in Table 17. Colony development was only slightly reduced when exposed to the low fungicide rate for two hr. Increased dosage (10 mg a.i./m^3 for two hr) reduced colony development significantly, but it was necessary to expose conidia to a high dosage (10 mg a.i./m^3 for six hr) before growth of the resulting colonies was completely inhibited (Fig. 9). This contrasts with 24-hr-old cultures which were killed after two hr of exposure to 1.8 mg a.i./m^3 (Table 12).

TABLE 15. Differential sorption of nuarimol by plastic and fiberglass.[a]

Treatment	Fungicide Effect[b]
Fiberglass Insulation	
Fumigated	2.7
Unfumigated	0
Polyethylene Sheeting	
Fumigated	0.3
Unfumigated	0
Fiberglass Sheeting	
Fumigated	0
Unfumigated	0
Check	0

a) Materials cut into 15 mm squares, exposed to 10 mg a.i./m^3 volatilized nuarimol for six hr at a vaporization temperature of 150°C and added to glass culture dishes each with four 24-hr-old cultures of rose powdery mildew. Fungicide effect ratings taken at 72 hr.

b) Fungicide ratings: 0 = no effect; 5 = colonies dead.

TABLE 16. The effect of nuarimol fumigation on germination on rose powdery mildew conidia.

Treatment[a]	Rate mg a.i./m^3	Exposure (hr)	Germination
Conidia fumigated after inoculation	1.8	2	101.7[b]
	10	2	99.5
	45	4	77.7[c]
Conidia fumigated before inoculation	10	2	101.5
	10	6	101.2
	45	4	87.8

a) Conidia were placed on leaf sections, counted, then fumigated or conidia were first fumigated then placed on leaf sections and counted. Treatments and corresponding untreated checks were paired leaf samples. Germination was rated 24 hr after treatment.
b) Values based on a minimum of 200 conidia per treatment; 50 per each of four replicated leaf sections. Presented as percent of untreated checks.
c) Significantly different from untreated check, p = .05.

TABLE 17. Colony development from conidia fumigated with
          nuarimol and transferred to unfumigated leaf tissue.

Exposure (hr)	Rate (mg a.i./m^3)	Colony Growth[a]		
		24	48	72
2	1.8	–	3.27[b]	3.75
0		–	3.33	3.87
			LSD (0.05) = 0.06	
2	10.0	2.13	2.78	3.27
0		2.41	3.30	4.03
			LSD (0.05) = 0.20	
4	10.0	2.27	2.46	2.82
0		2.39	3.31	3.75
			LSD (0.05) = 0.14	
6	10.0	1.61	1.61	1.61
0		2.25	3.21	3.65
			LSD (0.05) = 0.15	

a) Colony growth ratings were taken 24, 48 and 72 hr after
   treatment.   0 = no germination; 6 = normal sporulation.
   Each treatment and its corresponding untreated check are
   paired samples.
b) Ratings are means of 400 colony observations: 25 observations
   per each of four replications, repeated four times.
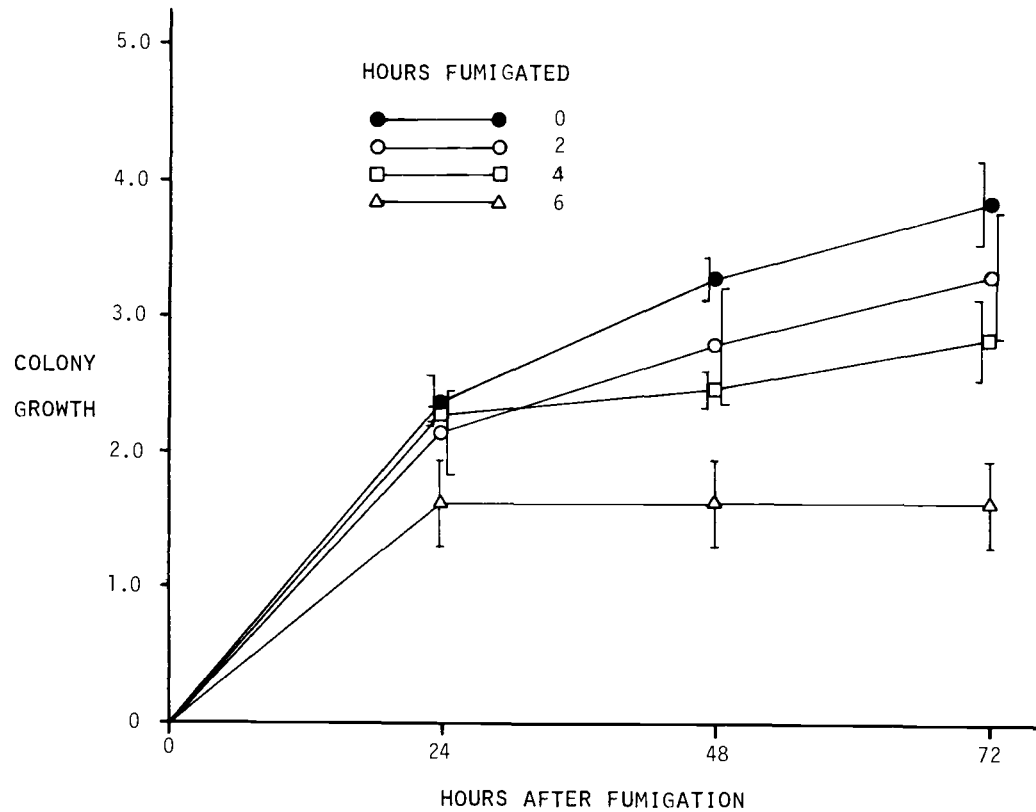
Figure 9. Colony development from conidia fumigated with nuarimol (10 mg a.i./m^3) for two, four, or six hr and subsequently transferred to untreated leaf tissue. Colony growth ratings: 0 = no germination; 5 = sparse sporulation. Vertical lines are standard deviations.

Colonies developing from conidia fumigated for two and four hr produced limited aerial hyphae and some hyphal tip swellings but growth continued past the swellings and colonies eventually sporulated. Aerial hyphae occasionally developed as colonies matured but the effect of the fungicide was obviously diminishing. In the 6-hr treatment hyphal growth did not continue beyond the swellings (Fig. 10).

Unfumigated or fumigated leaves were inoculated with unfumigated or fumigated conidia to determine the relative sorption of toxicant by the host and the pathogen (Table 18). The fungus was killed in both treatments in which leaves were fumigated but there was no significant difference between them. The effect of the fungicide on colony growth was small but significant when only conidia were fumigated.

The results of an experiment designed to examine the residual activity and translocation of nuarimol in fumigated whole plants are given in Table 19. The leaves inoculated at three days were immature and still tightly folded at the time of fumigation while those inoculated at seven days were in the primordial stage of development when fumigated. The fungicide remained highly active on plant surfaces for at least three days. Most colonies were killed, but those that continued to grow produced aerial, twisted hyphae and many irregular swellings. Surviving colonies were compact with restricted lateral growth and none developed to sporulation (Fig. 11). There was a slight fungicide effect on

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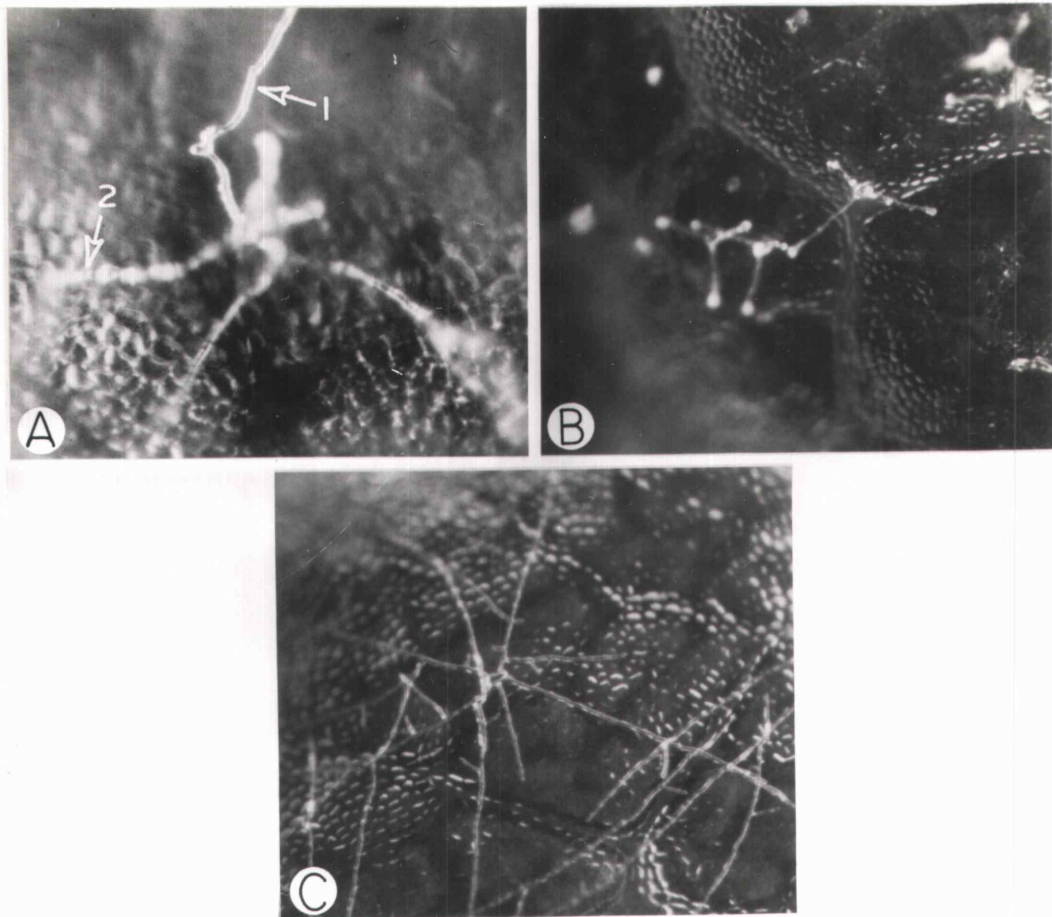
Figure 10. Difference in colony development from conidia fumigated
four hr and six hr with nuarimol. A. Colony developing
from conidium fumigated for four hr with nuarimol showing
(1) abnormal aerial hyphae and (2) hyphal strand continu-
ing growth beyond swelling (X160). B. Colonies developed
from conidia fumigated for six hr. Growth did not con-
tinue beyond hyphal tip swellings (X80). C. Untreated
check (X80).

TABLE 18. The effect of separate nuarimol fumigation of rose leaves and powdery mildew conidia on subsequent colony development.

Treatment[a]		Fungicide Effect[b]	Colony Growth[c]		Growth % of Check	
Conidia	Leaves	48 hr	48 hr	72 hr	48 hr	72 hr
X	X	5.00^d	2.01	2.01	60.1	52.4
	X	5.00	2.02	2.02	60.3	52.6
X		1.28	3.28	3.77	98.2	97.6
Untreated Check		0	3.34	3.86	–	–

a) Conidia and/or leaves were fumigated with 1.8 mg a.i./m^3 nuarimol at 150°C for two hr before conidia were applied to leaf surface.
b) Fungicide effect rating: 0 = no effect; 5 = colonies dead.
c) Colony growth rating: 0 = no germination; 6 = normal sporulation.
d) Ratings are means of 400 colony observations: 25 observations per each of four replications, repeated four times.

TABLE 19. Translocation of nuarimol in young rose tissue after 6 hr exposure to the volatilized fungicide.[a]

Days Between Fumigation And Inoculation	Days Between Fumigation And Evaluation	Fungicide Effect[b]
0	3	5.00
3	5	4.63
7	10	0.56

a) Potted rose plants (cv. Dwarf Crimson Rambler) fumigated six hr with nuarimol (10 mg a.i./m^3), volatilization temperature 150°C. Leaf sections were detached and mass inoculated as young leaves unfolded on the plant.
b) Fungicide effect rating: 0 = no effect; 5 = colonies dead.
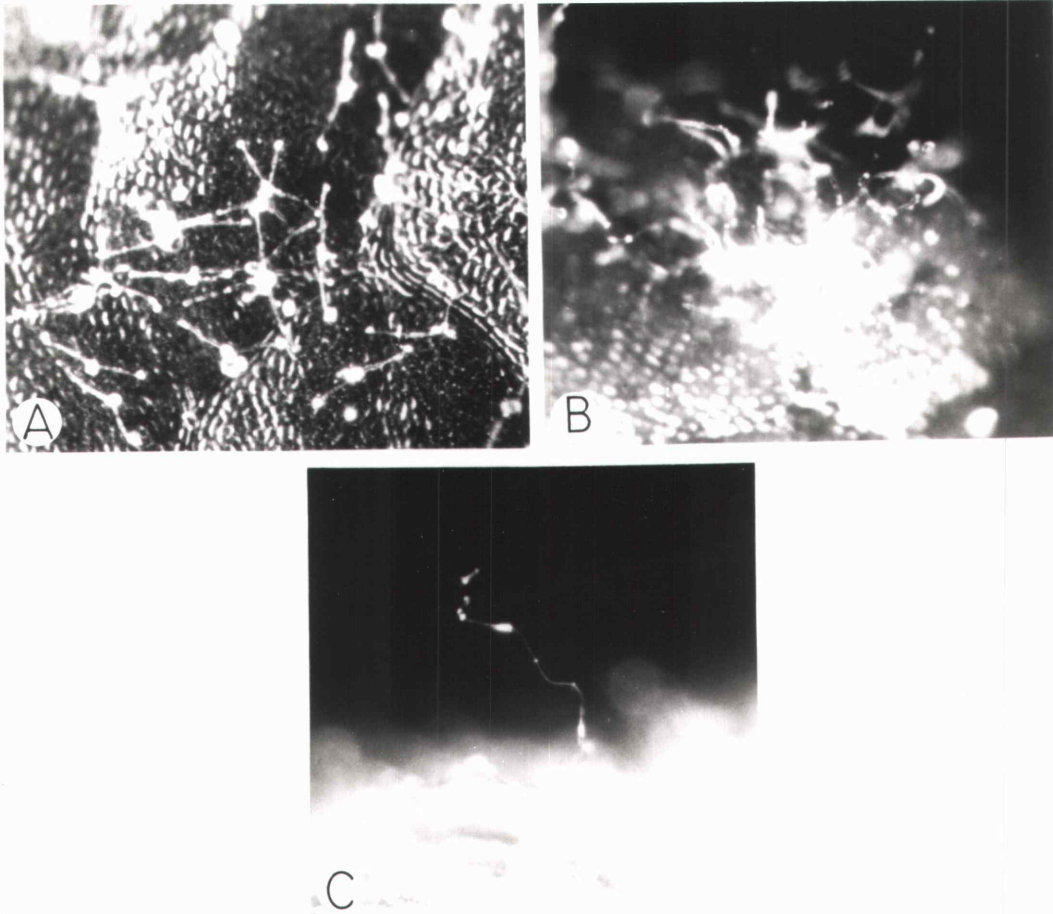
Figure 11. Colony development on rose leaves fumigated with
            nuarimol 72 hr prior to inoculation with untreated
            conidia. Photographed two days after inoculation.
            <u>A</u>. Colonies killed (X80). <u>B</u>. Surviving colony compact
            with aerial hyphae (X80). <u>C</u>. Twisted aerial hyphal
            strand (X80).

the powdery mildew in the leaves inoculated at seven days. A few hyphal tip swellings were visible but there was little evidence of decreased colony growth. Hyphae continued growth beyond the swellings with little inhibition (Fig. 12).

Volatile activity against rose powdery mildew is not limited to nuarimol. The experimental fungicides fenapanil and bupirimate were at least as effective as nuarimol in killing the fungus in the fumigation chamber at rates of active ingredients approximating those used in standard spray treatments (Table 20). All three fungicides killed young, actively growing colonies within 24 hr after fumigation. Bupirimate was unsatisfactory as a fumigant when heated to 105°C in greenhouse and growth chamber experiments but when the temperature was elevated to 170°C (Table 20) it was more effective as a fumigant than either nuarimol or fenapanil. Nuarimol and fenapanil have little or no activity against the germination of conidia, but no conidia germinated in the bupirimate treatments after ungerminated conidia were treated (Figs. 13 and 14). The growth of fumigated 24-hr-old colonies was significantly less for bupirimate than for either of the other two fungicides.

Ascocarp Induction

Sphaerotheca pannosa var. rosae ascocarps have been observed on 'Dwarf Crimson Rambler' roses only on the hypanthia, pedicels, and occasionally on stems near thorns. Fruit-set can be induced
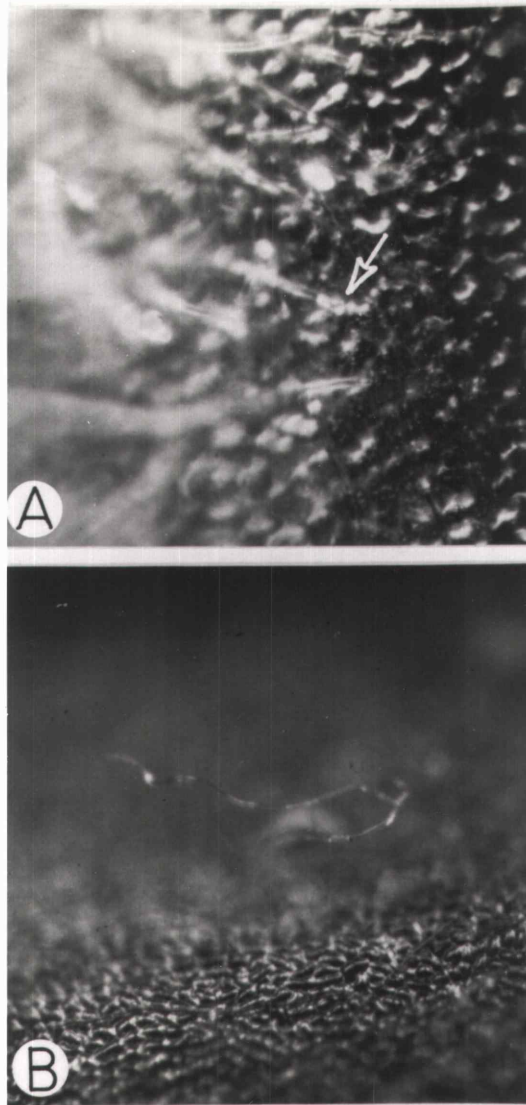
Figure 12. Fungus development from conidia transferred to leaf
tissue fumigated with nuarimol seven days previous.
A. Occasional hyphal tip swellings were present
(arrow) (X160). B. Aerial hyphae were evident (X80).

TABLE 20. The effect of volatilized fungicides on conidia and young colonies of Sphaerotheca pannosa var. rosae in an enclosed chamber.

Treatment	Rate (mg a.i./m^3)	Volatilization Temperature (°C)	Fungicide Effect[b] 24 hr	Colony Growth[b] 48 hr	Growth % of Control 48 hr
Fenapanil	15	150			
Ungerminated conidia			5.00	2.00	55.26
Control	0		0	3.62	
Fenapanil	15	150			
24-hr-old colonies			5.00	2.34	62.35
Control	0		0	3.75	
Bupirimate	50	170			
Ungerminated conidia			5.00	0	0
Control	0		0	3.53	
Bupirimate	50	170			
24-hr-old colonies			5.00	1.86	50.29
Control	0		0	3.69	
Nuarimol	10	150			
24-hr-old colonies			5.00	2.58	66.78
Control	0		0	3.86	

a) All fungicides volatilized for four hr. Colonies on detached leaf sections were 24 hr old at fumigation or conidia were applied to leaf sections immediately before fumigation.

b) Fungicide effect ratings were taken 24 hr after treatment. Colony growth ratings taken 48 hr after treatment. Each treatment and its corresponding untreated controls are paired samples. Ratings are means of 400 colony observations: 25 observations per each of four replications, repeated four times. All treatments statistically different from the corresponding untreated control (p = .05).
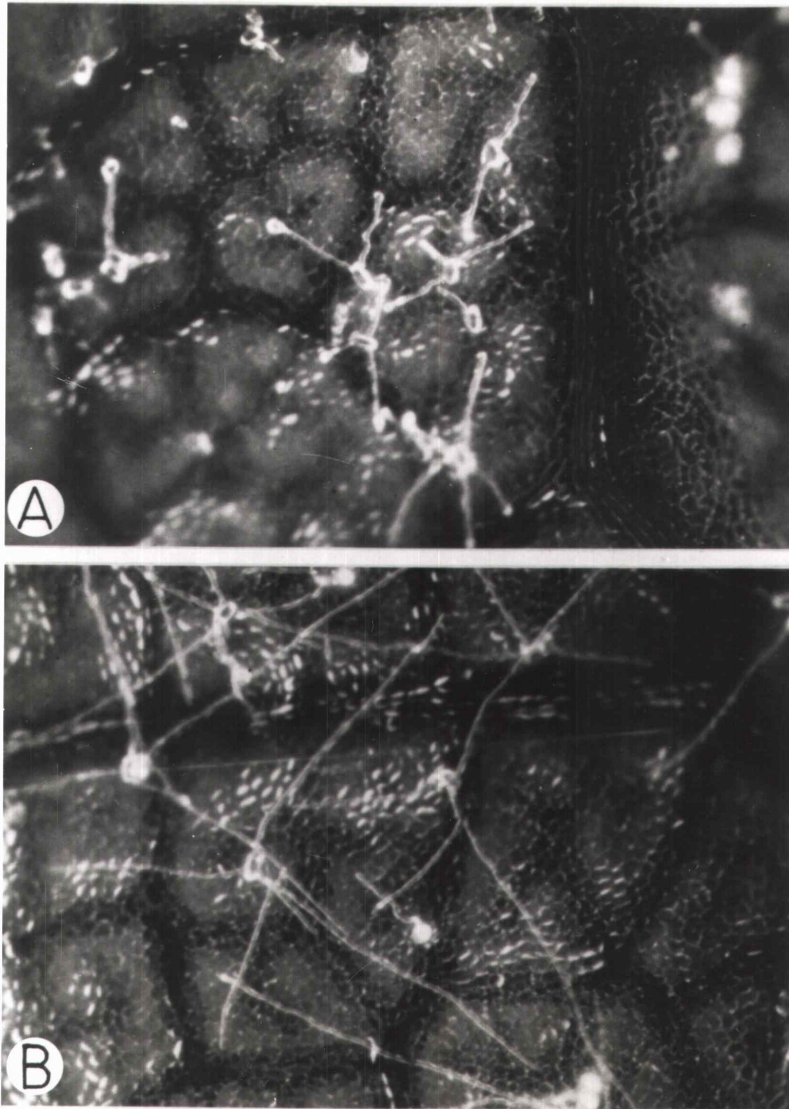
Figure 13. Effect of fenapanil volatilization on powdery mildew
colony development. A. Twenty-four-hr colonies killed
by fenapanil (X80). B. Untreated check (X80).
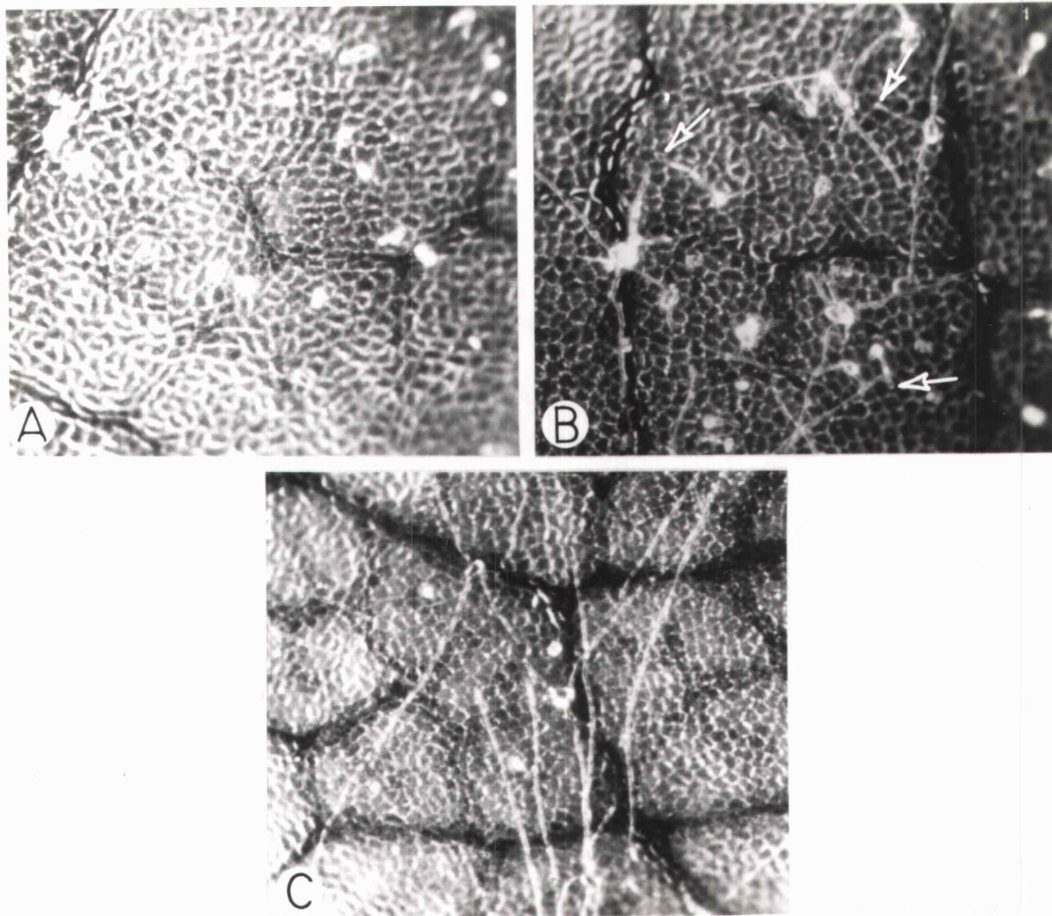
Figure 14. Effect of bupirimate volatilization on powdery mildew
conidia germination and colony development. A. No
germination of conidia when fumigated with bupirimate
(X80). B. Hyphal tip swellings in 24-hr colonies
killed by bupirimate were smaller than those killed
by nuarimol or fenapanil (arrows) (X80). C. Untreated
check (X80).

in certain plant species with auxins, in others with gibberellins and still others with cytokinins (36). Differential plant growth hormone-like responses on various plant parts in this rose cultivar suggest that these hormones may play a role in the development of ascocarps on the hypanthia (Fig. 15).

A wide range of hormone types and concentrations were tested but no ascocarps were produced on plants in any of the treatments or the untreated checks (Table 21). One experiment was conducted using a plant with the closely appressed, tan mycelium which is consistently observed just before ascocarps develop on hypanthia and pedicels of 'Dwarf Crimson Rambler'.

Six combinations of fertility and daylength were maintained for 45 days to test the environmental effects on development of ascocarps. Treatments were: (a) shadehouse - no fertilization, (b) shadehouse - weekly fertilization, (c) greenhouse - no fertilization, 15 hr daylength, (d) greenhouse - no fertilization, 11 hr daylength, (e) greenhouse - weekly fertilization, 15 hr daylength, and (f) greenhouse - weekly fertilization, 11 hr daylength. No ascocarps developed in any of the treatments. However, 37 days before the initiation of this experiment 12 plants of the same variety which were infected with powdery mildew in the greenhouse were moved to the shadehouse for use in an unrelated experiment. Immature cleistothecia were observed 23 days later on all 12 plants in the shadehouse while there were none on any of the many plants grown in the greenhouse.
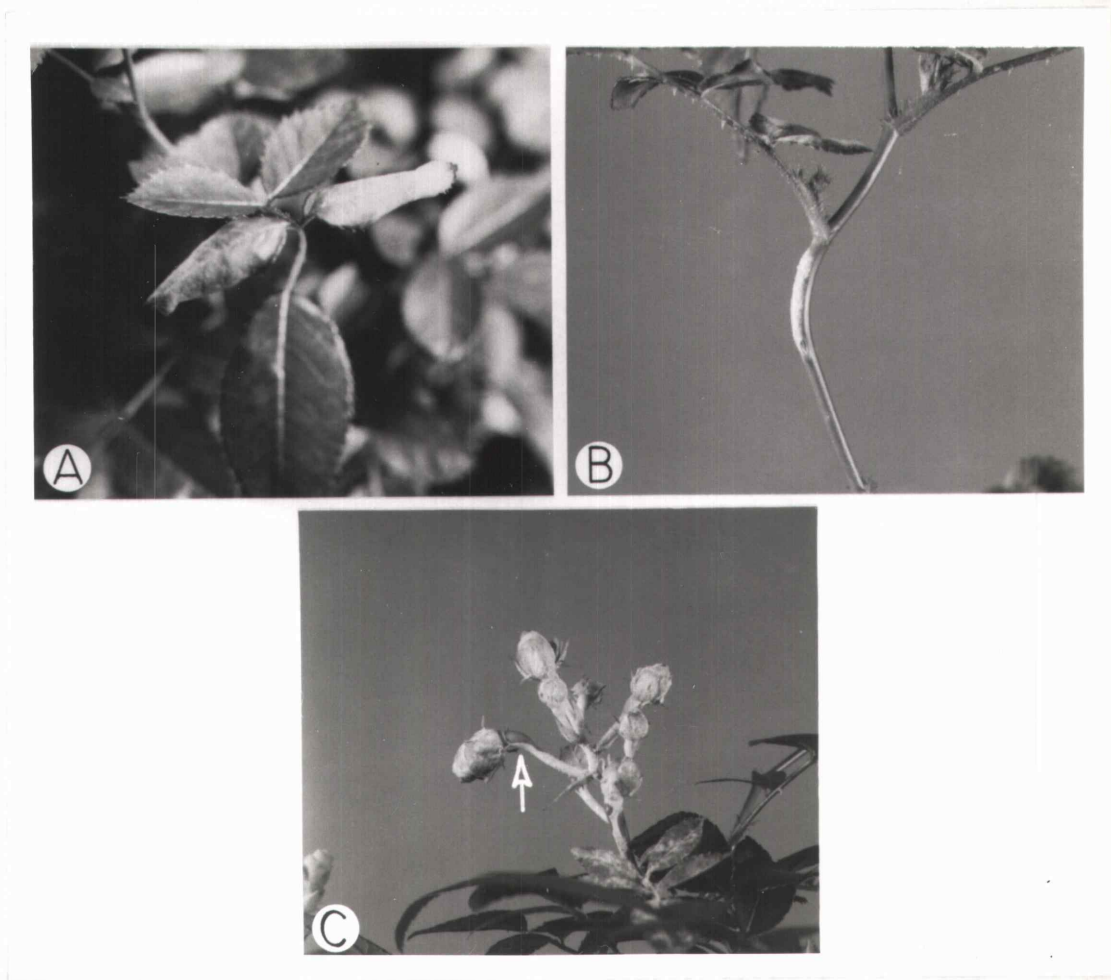
Figure 15. Differential growth of rose plant parts in response to
            infection by powdery mildew. A and B. Hyperplasia in
            rose leaf in response to infection by rose powdery
            mildew on the upper leaflet surfaces and rose stem
            in response to infection on a single side. C. Hypoplasia
            in an hypanthium in response to infection.

TABLE 21. Plant growth hormone treatments used to induce
           ascocarp development in _Sphaerotheca pannosa_
           var. _rosae_.

Pre-Treatment	Treatment[a]		
3 mo; 15 hr daylength; 17-27°C; flowers closed.	IAA: 10^{-3}M, 10^{-4}M, 10^{-5}M IBA: 10^{-3}M, 10^{-4}M, 10^{-5}M GA: 10^{-3}M, 10^{-4}M, 10^{-5}M Kinetin: 10^{-3}M, 10^{-4}M, 10^{-5}M		
3 mo; 15 hr daylength; 17-27°C; flowers 1/2 opened.	IBA: 10^{-3}M, 10^{-4}M, 10^{-5}M		
3 mo; 12 hr daylength; 11-21°C flowers closed.	IAA: 10^{-4}M IBA: 10^{-4}M		

a) All plants 100% infected at time of treatment.

Nine single-spore isolates were paired on plants in all possible combinations in individual isolation chambers and one plant was inoculated with all nine isolates. All cross inoculations were maintained for a minimum of 75 days. Weekly observations revealed normal disease development in all treatments, with no development of pannose mycelium or ascocarps. Microscopic examination of the mycelium on the hypanthia did not reveal any evidence of ascocarps or ascocarp initials.

DISCUSSION

Fumigation

Excellent control of rose powdery mildew can be obtained in greenhouses utilizing the volatile properties of nuarimol and fenapanil. Foliage and flower quality are improved and labor is greatly reduced over conventional spray applications which provide equivalent disease control.

The amount of fungicide available for disease control in the vapor phase is a function of (a) the chemical and physical nature of the fungicide, (b) temperature, (c) total surface area of the liquid phase exposed to the air, and (d) the length of time the fungicide remains at the proper temperature. An increase in the temperature of a liquid is accompanied by an increase in its vapor pressure, the particular values differing among materials. Proper volatilization temperature is of critical importance for powdery mildew control using sulfur (44,45) as its is with nuarimol and bupirimate.

At ambient temperature, nuarimol had no measured vapor phase activity (Table 13), but heating markedly increased activity. The greenhouse steam system used in the bupirimate suty was designed to maintain a steam pipe temperature of 105°C which was too low for effective volatilization. It was moderately effective in the laboratory at 105°C but when heated to 170°C or 185°C it was highly effective (Tables 2 and 20).

The liquid/air interface surface area is considerably reduced when the fungicide is placed in a small container compared with steam pipe application. Because the escape of a liquid molecule into the gaseous phase depends on a greater than average kinetic energy and nearness to the liquid/air interface, less time should be required to volatilize a given amount of fungicide as its exposed surface area increases. When the fungicide was heated in containers, it was reheated nightly because all the material did not vaporize in one heat application.

The data in Table 4 shows that the duration of heating is an important factor in fumigation efficacy. Reduced heating (1 1/2-2 hr) caused a less than acceptable level of control, while increased heating time (3-4 hr) resulted in excellent disease control. Although the chemical may completely vaporize it will not control the disease if the vents are opened and the toxicant permitted to escape before sufficient uptake by the fungus or the plant.

An understanding of the effect of these fungicides on particular stages of fungus development and the nature of their affinities to various materials and plant parts is essential to develop an effective control program in commercial greenhouses. Fumigation of the leaves with nuarimol plays a more important role in arresting the growth of the fungus than does fumigation of the fungus itself. Differential absorption of nuarimol by the host and the fungus is indicated. The data on conidial versus plant uptake of nuarimol (Tables 12,16,17,18) suggest that direct chemical uptake by the

fungus from the surrounding atmosphere is comparatively low in this

system. It was necessary to subject conidia to 10 mg a.i./m^3 for

6 hr before their growth on untreated leaf tissue was arrested;

a 28-fold increase in total dosage over that necessary to kill one

day old colonies (Table 17). Other supporting evidence is the lack

of difference in the observed fungicide effect and colony growth

when the leaves were fumigated and the conidia were or were not

fumigated (Table 18).

Haustoria are probably the primary absorptive organ of powdery

mildews. According to Bushnell and Gay (8) the key to the movement

of solutes from the host to the fungus lies in processes at the

interface between the haustorium and the cytoplasm of the host.

Staub et. al. (57), using scanning and transmission electron

microscopy, observed imprints of germ tubes, appressoria and hyphae

on the cuticular surface of host and nonhost plants after E. graminis

and E. cichoracearum were removed from the surface. Epicuticular wax

crystals were dissolved suggesting enzymatic secretions from these

structures. Lukens and Horsfall (42) hypothesized that lipophilic

organic fungicides may be systemic but not necessary physiologi-

cally transported through the active transport system of the plant.

Because they partition from water into oil, these fungicides may

partition from the surface water or residue into cuticular wax

and diffuse to distant parts of the cuticle. The fungus may contact

the toxicant while contacting this layer. Such movement of pesticide

through the cuticle may account for the observed movement of nuarimol

in leaves which were in the primordial stage during fumigation (Table 19).

Nuarimol and fenapanil inhibit the biosynthesis of ergosterol (18) which is the primary sterol component of fungal membranes. Conidia that germinate and establish an incompatible relationship with the host continue to actively absorb solutes (21,43). In this study, incompatible conidia consistently appeared turgid 72 hr after fumigation with both nuarimol and fenapanil (Fig. 8). Ungerminated conidia and those colonies which were growing rapidly at the time of fumigation were severely dessicated. Apparently both fungicides interfere with some growth process, probably membrane development, because actively growing colonies are the most sensitive to exposure, while incompatible conidia are not affected. The observation that bupirimate prevents conidial germination (Table 20 and Fig. 14) suggests that its biochemical mechanism of action is different from that of nuarimol and fenapanil.

Young, rapidly growing colonies are highly sensitive to nuarimol vapors (Table 12), but older colonies appear more resistant as indicated by the superior disease control on whole plants in the greenhouse and the laboratory on 4-day rather than 7-day intervals. Discontinuing treatment after a 7-day interval could allow old colonies to produce conidia and perpetuate the infection. This would be more likely in a situation where the infection in an entire greenhouse was very heavy as it was on the plants tested in these experiments. The fumigation interval must be short enough to prevent formation of new sporulating colonies before the next

treatment. Colonies may be protected within infected buds and produce viable conidia which begin new colonies on tissue developing after fumigation.

The long-term disease control obtained following nuarimol fumigation was particularly interesting (Table 3). One advantage of fumigation over spray application is more uniform distribution of the toxicant which is frequently not the case with spray application. This probably accounts for improved disease control at lower fungicide rates than with spraying. The result is a reduction in initial inoculum level (X_o) and a delay of the epidemic. If X_o is reduced to 0, no disease occurs until new inoculum enters the greenhouse. However, the slow disease progress in this house compared with the other three and the six mo delay before disease incidence was high enough to warrant further control measures cannot be attributed soley to the sanitation effect.

It is clear that nuarimol is sorbed in the vapor phase by several substances then released to provide a continuing effect on the fungus. Tested materials with specific activity were glass, polyethylene sheeting and fiberglass insulation. The chemical was probably sorbed by materials in the greenhouse structure and released over time to provide a lasting effect against the pathogen. The residual fungicidal effects in growth chambers after repeated fumigation with nuarimol provide supportive evidence for this hypothesis.

Systemic uptake by the plant may occur during repeated treatments and subsequent translocation of the toxicant could protect the new growth. Systemics are usually translocated upward in plants

(22). Because fenarimol has upward mobility (7) and is an analog

of nuarimol with nearly identical structure and properties (37,38),

it is likely that nuarimol has a similar translocation pattern.

Whole plants were shown to sorb enough nuarimol in a single 6-hr

fumigation to have an effect on the pathogen ten days after treat-

ment in leaves which were not developed at the time of fumigation.

Although slight systemic movement is evident, the activity is not

great enough to appreciably slow development of the fungus. It

is suggested that volatilized nuarimol provides longterm control

by combining sanitation effects, sorption and subsequent release

by components of the greenhouse structure and systemic activity.

The least important of the three factors is probably systemic

activity. No residual effect in the greenhouse was observed

with fenapanil.

No attempt was made to alter any practices or temperature regimes

that were normally encountered in each grower's operation, because

the purpose of these tests was to determine whether fumigation of

rose powdery mildew was effective in commercial greenhouses over a

wide range of conditions. Maintaining night temperatures at 15-18°C,

which is normal for rose culture, gave excellent disease control

with both nuarimol and fenapanil. Higher greenhouse temperatures

may aid in the dispersal of the toxicant more effectively throughout

the greenhouse or increase its efficacy against the fungus as is the

case with sulfur (44), but raising the night temperature would result

in more blossom production accompanied by an unacceptable reduction

in flower quality.

One of the major benefits of fumigation is greatly improved applicator safety compared with conventional spray application. The only handling of the pesticide is the addition of the concentrate to the fumigation container, after which the heating and subsequent ventilation of the greenhouse is accomplished by automation. The entire operation is completed at night during the absence of greenhouse workers.

In practice it is preferable not to volatilize fungicides from steam pipes because it is undesirable to apply heat during warm weather and residues would be volatilized whenever the pipes are heated, posing a hazard to greenhouse occupants. Heating fungicides in small containers eliminates these problems and allows greater temperature control flexibility.

The labor required to treat an entire commercial greenhouse is only a fraction of that required for a standard spray treatment. One person can accomplish in 20 minutes what would normally require two people working at least four hr. The data also indicate that better disease control can be obtained using less fungicide resulting in a final product of higher quality than is obtained by spraying.

The difference in disease control observed with triforine at two locations suggests development of field resistance in S. pannosa var. rosae to this fungicide. Growers have remarked that triforine does not control powdery mildew as well as it once did. Triforine resistance has been induced in the laboratory in Cladosporium cucumerinum but the resistant mutants were less virulent and

spores they produced showed a decrease ability to germinate (17),
a characteristic that would reduce fitness and survival in the
field.

The long residual control with nuarimol is desirable from the
grower's point of view since costs are greatly reduced, but in the
long-term this characteristic may be a disadvantage. Continued
selective pressure for tolerance in the pathogen could cause earlier
development of a resistant population. There are reported cases of
resistance to other pyrimidine fungicides (2). Resistance to both
ethirimol and dimethirimol in cucumber powdery mildew in Europe
made these chemicals totally ineffective in Holland until stabil-
izing selection in the pathogen once again permitted their occasional
use.

Cross-resistance to two or more fungicides with the same bio-
chemical mode of action has been reported in certain fungi (54,55).
Strains of Cladosporium cucumerinum that are resistant to triarimol,
a closely related predecessor of nuarimol, are cross-resistant to
triforine, but this resistance has not been encountered in the field.
Both compounds are ergosterol biosynthesis inhibitors. Simply
because two fungicides have similar chemical structures or are
closely related in their biochemical modes of action does not mean
that cross-resistance to them will necessarily develop.

Control programs that reduce or prevent the buildup of resistant
pathogen populations are more desirable than remedial measures which
include cessation of treatment with a specific fungicide. Fungicide

combinations, either as a mix or in a sequential application program with other fungicides with different mechanisms of action may solve the problem or prevent its development (18). Ebben and Spencer (20) found that benomyl and dimethirimol used in combination against cucumber powdery mildew prevented development of strains resistant to both compounds.

In order to prevent development of resistant populations through the use of volatile fungicides in commercial greenhouses, it is imperative that fungicides be screened for their volatile activity, their mode of action and their potential for selecting resistant populations. Combination systems could then be developed to allow indefinite use of this application method. An understanding of the problem will prevent the development of resistant populations. Without such understanding, the powdery mildew population would be subjected to a high dosage of fungicide over long periods of time; the very conditions that exert strong selective pressure toward resistant populations.

Investigations should be conducted to determine if presently registered fungicides are effective in the vapor phase against powdery mildew and other foliar diseases. The potential of this application method for widespread use in the greenhouse industry is promising.

Ascocarp Induction

The development of the sexual stage in S. pannosa var. rosae is likely a function of heterothallism in the fungus and dependent

on a particular combination of environmental conditions. In addition ascocarp development may depend on the ability of two required mating types to develop on a host or the involvement of a necessary host factor found only in certain cultivars. The observation that ascocarps developed on plants removed from the greenhouse to the shadehouse but not on those remaining in the greenhouse suggests that proper fungal mating types were present and that temperature plays an important role.

Solving the problem may require a more specific approach rather than the broad one used in this study. Culture of powdery mildew colonies from ascospores would increase the probability of isolating the required mating types assuming heterothallism for S. pannosa var. rosae. Because the sexual stage of rose powdery mildew occasionally occurs on cv. Dwarf Crimson Rambler it would be a likely host to study the sexuality of the fungus.

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