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Control of the Black Mold Fungus *Chalaropsis Thielavioides* Peyr. on Manetti Rose

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INTRODUCTION

THE black mold fungus *Chalaropsis thielavioides* Peyr. has been reported on Manetti rose by Masse and Longree (7), Longree (6), and Baker and Thomas (1). This fungus was found on Manetti rose in Oregon in 1938 and has been reported in Washington (3). It has been reported on other host plants, namely lupine (8), walnuts (4) and Chinese elm (5). Losses result from this organism infecting the roots of lupine and Chinese elm, and from its development as a wound parasite on walnuts and Manetti rose. Since *C. thielavioides* can attack such a group of distinctly unrelated species of plants its economic importance is readily recognized.

Considerable losses have been sustained by Oregon growers of Manetti rose because of attacks by *C. thielavioides*. Its presence on rooted Manetti cuttings makes this stock unfit for graft bench propagation in greenhouses. It grows on the ends of Manetti cuttings and on wounds left by disbudding, resulting in poor callus formation and subsequent poor root formation. It develops under bud shields on field-budded Manetti, thus preventing callus formation and causing the bud to die. These losses were so serious that the work herein reported was initiated. Undoubtedly this organism will continue to be a problem since it attacks such a wide range of host plants and has become quite generally distributed.

SYMPTOMS AND DEVELOPMENT OF THE DISEASE

C. thielavioides on Manetti rose first appears as a white granular growth of mold that rapidly spreads over cut and bruised areas (Figure 1A). This growth is composed of some mycelium, but mostly it consists of endoconidiophores and endoconidia (Figure 2A). Soon this growth begins to darken and becomes very black. This black color is due to the formation of an abundance of single-celled, dark, thick-walled, circular macroconidia (Figure 2B). The mycelium seldom enters more than a few rows of cells below the injured surface. The fungus is readily recognized by microscopic examination because of the two types of characteristic spores.

The disease is perpetuated by bringing contaminated plants into storage houses where conditions are ideal for the fungus to develop and produce spores. The dust, air, and equipment soon become thoroughly contaminated with spores, which germinate and develop as soon as they come in contact with injured Manetti. Manetti cuttings for the next year's crop of roses are usually cut and disbudded in these same houses and all cut surfaces may become infected before the cuttings are planted. If the infection progresses rapidly all callus formation is prevented and the cutting does not take root. Frequently the

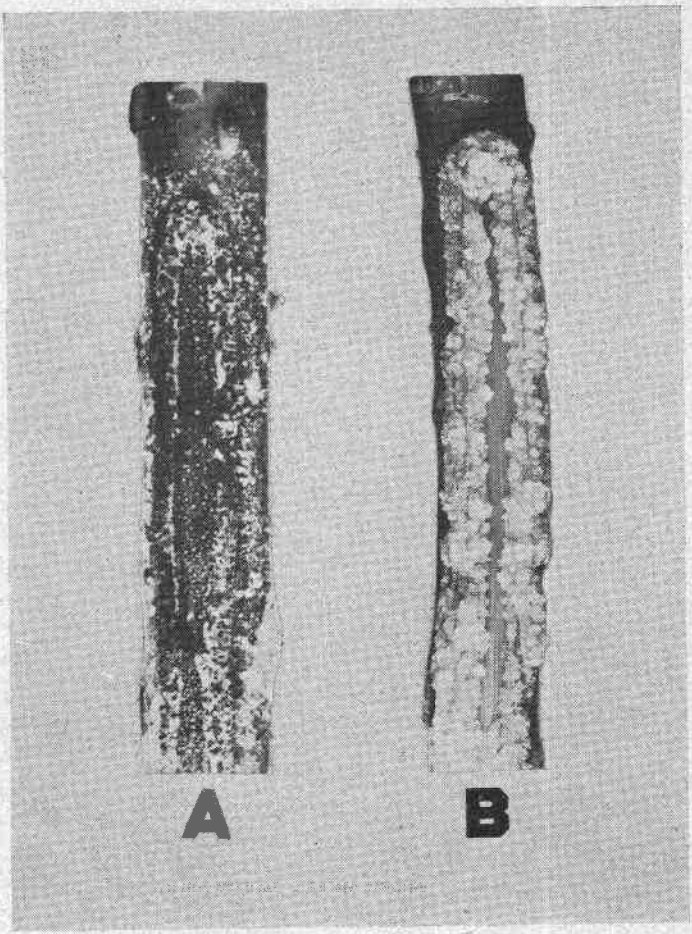

Figure 1. Injured portions of two Manetti rose cuttings. *A:* Inoculated with spores of *C. thielavioides* and showing black mold growing over injured portion. There is no callus development. *B:* Not inoculated. Note abundant callus formation along cambium area.

infection does not entirely prevent the cutting from rooting and subsequent growth of the cutting prevents further development of the fungus. Callus formation often grows over and completely covers patches of spores. These remain dormant until exposed by cutting into these areas during budding or grafting operations. Then the fungus rapidly grows over the injured area, and prevents callus formation. Manetti grown for dormant rooted cuttings become infected in the same manner.

EFFECT OF FUNGICIDAL MATERIALS ON BLACK MOLD

The major portion of this study has been the testing of different fungicides to determine their effectiveness in killing black mold spores. The following laboratory method was devised which greatly facilitated this work. Manetti rose stems were cut in pieces 5 inches long and about one third of the upper end of these cuttings were slashed with a knife to remove a strip of bark. This injured area was then painted with a spore suspension of *C. thielavioides* by using a camel's hair brush. The cuttings were allowed to dry before further treatment so that the spores would become fixed and not wash off. The fungicide to be tested was prepared in proper dilutions and placed in quart Mason jars. Bundles of five Manetti cuttings were then dropped into these fungicides and left for the treatment period. They were then removed and dried and the five cuttings placed separately in 6-inch test tubes with about 1 inch of sterile water. The tubes were plugged with cotton and placed in racks where they could be observed for black mold development (Figure 3). Final records were made 2 to 3 weeks later. The amount of black mold development was used as a measure of the effectiveness of the fungicide. The amount of callus development on the cut area and the length of shoot growth were used as an index of fungicidal injury. Five cuttings were used for each treatment and many of the treatments were repeated at least once.

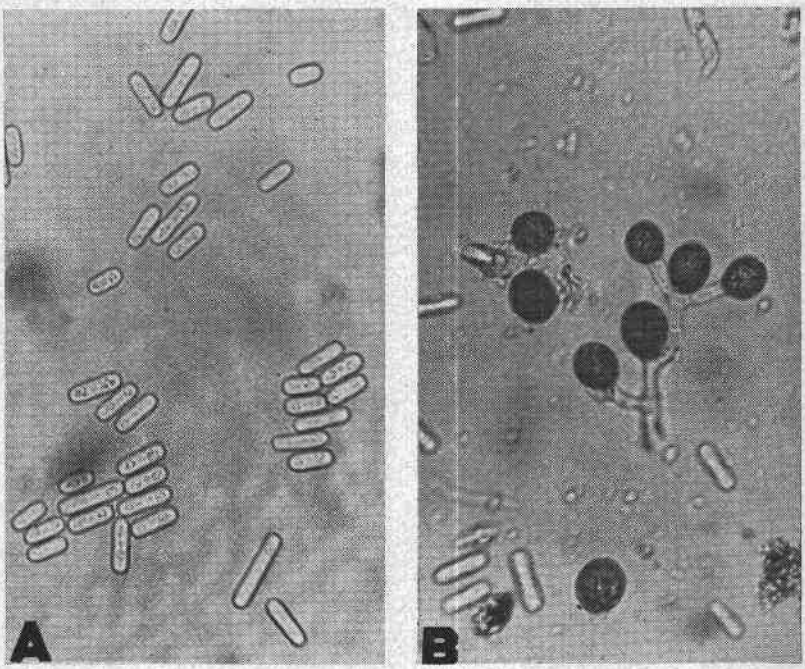

Figure 2. Photomicrograph of characteristic spores of *C. thielavioides*. *A:* Microconidia or endospores; *B:* Macroconidia.

The fungicidal values found for the different chemicals tested by this method are summarized in Tables 1 and 2. The outstanding feature of these results is the extremely high tolerance of these spores on host tissue to fungicidal materials. Black mold developed on Manetti cuttings after a two-hour treatment with mercuric bichloride solution of 1-1000. Spores germinated after all the color had been removed from the cell walls by clorox. An hour soak in a dormant lime sulphur did not kill the spores. Elegetol was relatively ineffective. Soaking the cuttings in formaldehyde diluted 1-150 for two hours did not kill all of the spores. Some of these spores might have been protected by air pockets or by some other factor that prevented the chemicals from coming in direct contact with the spores. Nevertheless the fact still remains that growth developed after such treatments, and these laboratory conditions were much more favorable than could be expected in a field treatment.

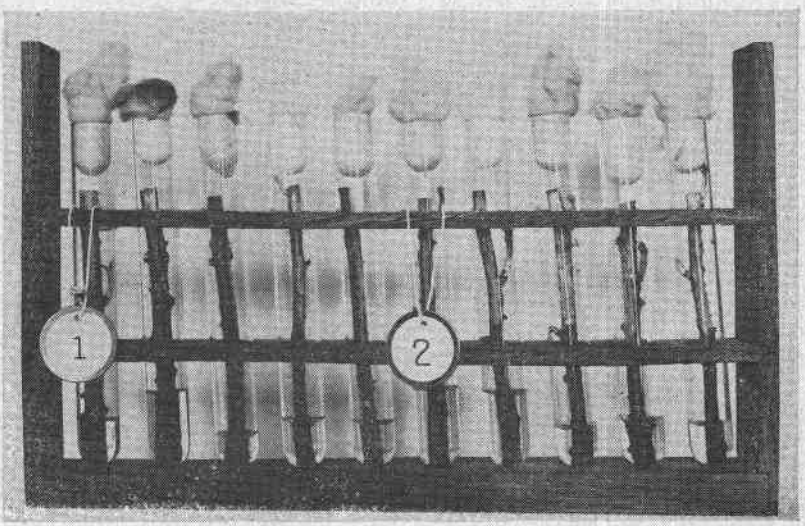

Figure 3. A rack of test tubes with the Manetti cuttings as they appeared near the end of the test period. Note the new growth developing on a number of the cuttings.

The efficiency of this method for indexing the fungicidal strength of different chemicals is shown by the tables. With many of the chemicals the time interval and concentration were closely related to the degree of mold development. For instance, Semesan in Table 2 shows the following correlation: 1 pound in 25 gallons for 10 minutes reduced growth only slightly, 1 pound to 15 gallons for 10 minutes reduced mold growth about 50 per cent, 1 pound in 10 gallons reduced growth to a trace, while 1 pound in 5 gallons for 10 minutes checked all growth. Likewise, 1 pound in 25 gallons for 10 minutes reduced mold growth only slightly, a 30 to 60 minute treatment with the same solution reduced it to about 50 per cent, a two-hour treatment reduced growth to a trace, while a four-hour treatment checked all mold growth.

Table 1. The Effects of Miscellaneous Chemicals on Black Mold Development[1]

Chemical	Concentration	Growth after treatment of intervals of							
		1 minute	10 minutes	30 minutes	60 minutes	120 minutes	240 minutes	480 minutes	1440 minutes
Elegetol	2%		++++	++--	++--	----			
	1%			++++	++++	+++-			
$KMnO_4$	1 lb.-25 gal.		++++	++++	++++	++++			
Mercurochrome	.2%	++++	++++	++++	++++	++++			
Bordeaux	10-10-50	+---							
Chloropicrin	10% of sat. soln.		++++	++--	+---	----			
Lime sulphur	1 to 8			++++	++++				
Clorox	10%				++--	+---	+---		
Acetic acid	0.1%					++++			
	1.0%					+---			
	5.0%				----	----	----		
Copper sulphate	.001%					++++			
	.01%					++--			
	.1%				----	----	----		
Formaldehyde	1 to 150		++++	++++	+---	+---			
	1 to 320			++++	++--	++--	+---		
Cresol	1.0%				----	----			
	0.2%				+++-	+++-			
	0.1%						++++	++++	++++
Supergermite	2.0%			----	----	----			
	1.0%			----	----	----			
	0.5%			+---	+---	----			
	0.2%				++--	+---			
	0.1%				++++	+++-	++--	++--	----
Malachite green	0.05%				+---	+---	+---	+---	----
	0.033%					+---			
	0.025%					+---			
	0.020%				+---	+---	+---	+---	+---
	0.010%				++--	++--	+---	+---	+---
Mercuric chloride	0.1%	++++				++--			
	0.05%			+---	+---	+---	----		
	0.025%					++--	+---		
	0.020%	++++				++--			
Mercurous chloride	2 oz.-1 gal.	++--							

[1] ++++ indicates no appreciable reduction of black mold growth by treatment, ++-- indicates 25-50 per cent reduction of mold growth, +--- indicates 50-95 per cent reduction of mold growth, +--- indicates mold growth reduced to trace, and ---- indicates no mold growth after three weeks incubation.

Table 2. The Effect of Mercury Compounds on Black Mold Growth[1]

Chemical	Concentration	Amount of growth after treatment interval of					
		1 minute	10 minutes	30 minutes	60 minutes	120 minutes	240 minutes
Spergon	1 lb.-5 gals.		++++	++++	++++	++++	
Thiosan	1 lb.-5 gals.		++++	++++	++++	++++	
DuBay 1286A	1 lb.-25 gals.					++++	
Barbak III	1 lb.-25 gals.		++++	++++	++++	++++	
Barbak C	1 lb.-25 gals.		++++	++++	++——	+———	
	1 lb.-50 gals.		++++	++++	++++	+———	
	1 lb.-100 gals.		++++	++++	++++	++——	
Corona PD 7	1 lb.-25 gals.		++++	++++	++——	+———	
	1 lb.-50 gals.		++++	++++	++++	+++—	
Special Semesan	1 lb.-25 gals.					+———	
	1 lb.-50 gals.					+———	
	1 lb.-100 gals.					++——	
Semesan Bel	1 lb.-10 gals.		+———	+———	————	————	
	1 lb.-20 gals.		++——	+———	+———	+———	
Semesan	1 lb.-5 gals.		————	————	————	————	
	1 lb.-10 gals.		+———	————	————	————	
	1 lb.-15 gals.		++——	+———	+———	————	
	1 lb.-20 gals.		+———	+———	————	————	
	1 lb.-25 gals.		++——	++——	++——	+———	————
	1 lb.-50 gals.					++——	
	1 lb.-100 gals.					++——	
Semesan Jr.	1 lb.-10 gals.		————	————	————	————	
	1 lb.-25 gals.		+———	————	————	————	
	1 lb.-50 gals.		+———	+———	————	————	
2% Ceresan	1 lb.-25 gals.	————	————	————	————	————	
	1 lb.-50 gals.		————	————	————	————	
	1 lb.-100 gals.		+———	————	————	————	
	1 lb.-200 gals.		+++—	++——	+———	————	
	1 lb.-300 gals.			++——	+———		
	1 lb.-400 gals.		++++	++++	+———	+———	
New Improved Ceresan	1 lb.-50 gals.		————	————	————	————	
	1 lb.-100 gals.		————	————	————	————	
	1 lb.-200 gals.		+———	————	————	————	
	1 lb.-300 gals.			————	————		
	1 lb.-400 gals.		++——	+———	+———	+———	
	1 lb.-500 gals.		++——	++——	+———		
DuBay 1155HH	1 lb.-25 gals.	————	————	————	————	————	
	1 lb.-50 gals.	————	————	————	————	————	
	1 lb.-100 gals.	+———	————	————	————	————	
	1 lb.-200 gals.	+———	+———	————	————	————	
	1 lb.-400 gals.		++——	————	————	————	
	1 lb.-500 gals.		++——	++——	+———		

Table 3. The Effect of Adding a Wetting Agent to the Fungicidal Material[1]

Fungicide[2]	Concentration	Wetting agent	Amount of growth after treatment interval of				
			1 minute	10 minutes	30 minutes	60 minutes	120 minutes
Semesan	1 lb.-25 gals.	Vatsol					+
	1 lb.-25 gals.	Ortho					+
	1 lb.-25 gals.	None					+
2% Ceresan	1 lb.-200 gals.	Wettalene		+	+	+	+
	1 lb.-200 gals.	B1956		+	+	+	+
	1 lb.-200 gals.	S.E.C. Oil		+	+	+	+
	1 lb.-200 gals.	Fluxit		+	+	+	+
	1 lb.-200 gals.	None		+	+	+	
Malachite green	1 to 5,000	Vatsol					+
	1 to 5,000	None					+
	1 to 10,000	Penetrol				+	+
	1 to 10,000	Summer oil					+
	1 to 10,000	Ortho					+
	1 to 10,000	None				+	+

[1]See footnote for Table 1 for key to table.

[2]The fungicides in Tables 1 to 3 listed under trade names are prepared by the following companies: Elegetol, Standard Agricultural Chemicals Inc.; Chloropicrin, Innis, Speiden and Company; Clorox, Clorox Chemical Company; Supergermite, Standard Oil Company; Spergon, United States Rubber Company; Thiosan, DuBay 1286A, Semesan Bel, Special Semesan, Semesan, Semesan Jr., 2% Ceresan, New Improved Ceresan, DuBay 1155HH, Du Pont Semesan Company; Barbak III, Barbak C, American Cyanamid and Chemical Corporation; Corona PD 7, Pittsburg Plate Glass Company; the wetting agents and spreaders used in Table 3 were prepared by the following companies: Vatsol, American Cyanamid and Chemical Corporation; Summer-oil, Sherwin Williams Paint Company; Penetrol, Kay-Fries Chemicals, Incorporated; Ortho fungicide adhesive, California Spray and Chemical Company; Fluxit, Colloidal Products Corporation; S.E.C. Oil and B1956, Rohm Haas Company.

Potassium iodide has been shown (9) to increase the fungicidal efficiency of mercuric chloride. Table 4 shows the effect of adding potassium iodide to mercuric chloride, mercurous chloride, and Semesan. In all instances the fungicidal activity of these materials in checking the growth of black mold was noticeably increased by addition of potassium iodide.

Table 4. THE EFFECT OF ADDING POTASSIUM IODIDE TO MERCURIAL FUNGICIDES

Fungicide	Concentration	Per cent KI	Amount of growth after treatment interval of			
			1 minute	30 minutes	60 minutes	120 minutes
Mercuric chloride	1-1000	.25%	+			
	1-1000	None	+			
	1-1200	.25%	+			
	1-1200	None	+	+	+	+
	1-2000	.25%	+	+	+	
	1-2000	.50%	+			
	1-2000	1.00%	+			
	1-2000	None	+	+	+	+
	1-3000	.25%	+			+
	1-3000	.50%	+			
	1-4000	.25%	+			+
	1-4000	.50%	+			
	1-4000	None	+			+
	1-5000	.25%	+			+
	1-5000	.50%	+			
	1-5000	None	+			+
Mercurous chloride	2 oz.-1 gal.	.25%	+			
	2 oz.-1 gal.	None	+			
	1-250	.25%	+			
	1-250	.50%	+			
	1-250	1.00%				
	1-500	.25%	+			
	1-500	.50%	+			
	1-1000	.25%	+			+
	1-1000	.50%	+			+
	1-1000	1.00%	+			+
Semesan	1 lb.-25 gals.	.25%	+			
	1 lb.-25 gals.	None	+	+	+	+
	1 lb.-50 gals.	.25%	+			
	1 lb.-50 gals.	None				+
	1 lb.-100 gals.	.25%	+			+
	1 lb.-100 gals.	None				+

THE TOLERANCE OF MANETTI CUTTINGS TO FUNGICIDAL MATERIALS

The laboratory method used in the foregoing tests of fungicidal materials also afforded an opportunity to determine tolerance of plant tissue to these different chemicals. Untreated Manetti cuttings placed in these test tubes and held for two to three weeks produced prominent rolls of white callus growth all along the injured area (Figure 1B). Also the buds would break dormancy and start to grow. Manetti cuttings treated with fungicides showed varying degrees of callus formation from no development to normal callus formation. Likewise the buds were so injured that they failed to grow or the growth was much reduced. In cases of severe injury necrosis of the tissue also was evident.

None of the chemicals tested prevented all growth of black mold without some injury to the Manetti cuttings. Several different fungicides showed little injury to the cuttings, when growth of the mold was restricted to a trace, but

the injury factor increased rapidly if stronger treatments or longer treatments were tried. This indicates that the tolerance of host and spores to these various disinfectants is very much the same.

Of all the materials tested, 2 per cent Ceresan as it approached its effective range caused less injury to the Manetti cuttings than other materials. Semesan caused very little injury up to reduction of the mold to a trace, but if treatments were sufficient completely to control the mold considerable injury became apparent. Semesan Jr., New Improved Ceresan, and DuBay 1155H were very effective in checking mold growth, but the injury factor also was greater than with 2 per cent Ceresan. The addition of wetting agents increased the injury to the cuttings. Likewise, increasing the efficiency of mercurials with potassium iodide increased the injury to Manetti. Chloropicrin, acetic acid, copper sulphate, formaldehyde, cresol, supergermite, Malachite green, and mercuric chloride all were very toxic to Manetti cuttings as they approached their effective range.

CORRELATION OF LABORATORY METHODS WITH FIELD PLANTINGS

Several of the more promising fungicides were selected from the laboratory studies and tested in field plots for their effectiveness in controlling black mold. One hundred Manetti cuttings were made for each material to be tested. These were disbudded as for nursery planting, dipped in a suspension of spores of *C. thielavioides,* and allowed to dry. They were then treated in the test fungicide and planted in 4 replications of 25 cuttings each in a field plot. In the fall the plants were dug, counted, graded, and then stored in a moist place suitable for black mold development. Later the amount of black mold was recorded. The results of these tests are given in Tables 5 and 6.

Table 5. 1943 FIELD TRIALS, CHEMICAL TREATMENTS OF CUTTINGS

	Rank and treatment	Cuttings rooted	Cuttings No. 1	Degree of black mold infection[1]
		Per cent	*Per cent*	
1	Semesan Jr. 1-50 1 hr. + IBA[2]	64	48[3]	+———
2	DuBay 1155HH 1-300 1 hr.	58	47	+———
3	Clean check + IBA	56	42	+———
4	2% Ceresan 1-100 1 hr.	57	40	++——
5	DuBay 1155HH 1-300 1 hr. + IBA	54	40	+++—
6	Clean check	54	39	+———
7	DuBay 1155HH 1-400 1 hr.	47	39	+—+——
8	2% Ceresan 1-200 1 hr.	49	38	+—+—+
9	5% Ceresan 1-300 ½ hr.	47	32	++——
10	5% Ceresan 1-300 ½ hr. + IBA	42	32	++——
11	5% Ceresan 1-400 1 hr. + IBA	53	29	++——
12	2% Ceresan 1-100 1 hr. + IBA	37	25	++——
13	5% Ceresan 1-200 ½ hr. + IBA	35	21	++——
14	Malachite green .025% 24 hrs.	50	20	++——
15	Semesan Jr. 1-50 1 hr.	41	16	+———
16	5% Ceresan 1-200 ½ hr.	28	13	++——
17	Malachite green .05% 24 hrs.	35	13	++——
18	Malachite green .05% 24 hrs. + IBA	26	12	+++—
19	5% Ceresan 1-400 1 hr.	29	10	+———
20	Black mold check	1	1	+++++

[1]See footnote for Table 1 for key.

[2]+ IBA cuttings given a 24-hour soak in a 1-25,000 Indole Butyric acid solution before planting.

[3]Per cent No. 1 is the number of plants with a uniform well developed root system and showed no injury from either chemicals or black mold.

Because of the infectious nature of this disease due to its abundant spore formation it was difficult to determine accurately the effect on mold eradication by the treatments. Several of the uninoculated checks developed a trace of black mold when stored and all of the treated lots had some mold. That the amount of mold on the cuttings treated with the stronger fungicides was greatly reduced and that the number of rooted plants was increased by these treatments are clearly shown in Table 5.

In 1944 several additional precautions were taken to prevent one lot of cuttings from becoming infected with spores from another lot. The results shown in Table 6 indicate that, since both check lots remained free from black mold, the mold that developed on the other lots was due to failure of the fungicide to kill all the spores before the cuttings were planted. In those lots showing a low percentage of rooting, some of the injury resulted from the fungicide and some from the lack of control of the black mold.

Indole butyric acid was used in an attempt to overcome some of the injury caused by using too strong a fungicide. Although this hormone increased the percentage of rooting to some extent, it is doubtful whether this would be of much practical help.

Table 6. 1944 FIELD TRIALS, CHEMICAL TREATMENTS OF CUTTINGS[1]

Rank and treatment	Cuttings rooted	Cuttings No. 1	Degree of black mold infection
	Per cent	*Per cent*	
1 Clean check + IBA	100.0	100.0	——
2 DuBay 1155HH 1-300 1 hr. + IBA	98.7	96.0	+++—
3 Clean check	97.3	93.3	——
4 2% Ceresan 1-200 2 hrs.	97.3	93.3	++—
5 2% Ceresan 1-100 1 hr.	98.7	85.3	++-+—
6 5% Ceresan 1-400 1 hr. + IBA	100.0	82.6	++—
7 DuBay 1155HH 1-300 1 hr.	96.0	82.6	++-+—
8 Semesan Jr. 1-50 1 hr. + IBA	93.3	80.0	+—
9 2% Ceresan 1-100 2 hrs. + IBA	89.3	78.6	++—
10 5% Ceresan 1-400 1 hr.	93.3	78.6	++—
11 Black mold check	87.0	66.0	+++-+
12 Semesan Jr. 1-50 1 hr.	98.7	57.3	+—
13 2% Ceresan 1-100 2 hrs.	74.6	41.3	——

[1]See footnotes for Table 5.

DISCUSSION

These studies have shown that chemical treatments alone will not eradicate black mold from a planting of Manetti roses. No treatment is known whereby infected rooted cuttings of Manetti could be treated in such a manner as to kill all spores. Spores covered with earth or buried under callus tissue could not be killed. It is doubtful whether even all of the surface-borne spores could be killed without seriously injuring the host. Chemical treatment of infected Manetti cuttings before planting increased the percentage of rooting and reduced the amount of spores present for further infections, but it did not completely eradicate the disease.

The reported control of black mold on walnuts (4) and on Manetti (2) by chemical treatment can be explained only on the supposition that most of the spores were washed from the stock by this treatment and the germination of the others retarded long enough for callus formation to take place. Manetti cuttings treated with several different chemicals developed no mold the first week and in some instances the trace infections did not develop until after two

to three weeks. The thin-wall fast-germinating microconidia are probably easily killed, but the slow-germinating thick-walled macroconidia are much more resistant to the fungicides and begin to germinate the second and third weeks. The resistance of these macrospores probably explains why traces of black mold were found on many of the field plants, even though the cuttings had been given treatments that prevented mold development for three weeks in the laboratory.

The control of black mold is going to depend on preventing infection by sanitary measures. This should not be too difficult. Since this is a wound parasite there is very little chance of Manetti mother blocks becoming infected. If clean Manetti is used for making cuttings and they are made in a clean storage house with clean equipment they should remain free from the disease. Since the spores could probably remain viable in the soil for some time, the cuttings should be planted in soil not previously used for Manetti.

SUMMARY

Chlaropsis thielavioides is becoming of increasing economic importance. It has been reported as affecting walnuts, lupines, Chinese elms, and Manetti roses. These reports have come from Italy, England, and in the United States from New York, Illinois, Oklahoma, California, Washington, and Oregon.

Studies on the control of this fungus on Manetti rose are reported in this paper.

Losses are caused by poor stands of cuttings, low grade plants with poor root development, low percentage of live buds of field-budded Manetti, and the production of infected rooted cuttings that are not suitable for greenhouse grafting.

The chief source of infection was found to be the spores in the dust and other debris in storage houses where the plants are handled and the cuttings prepared for planting.

A study was made of the tolerance of the spores of *C. thielavioides* to 28 different fungicidal materials. Different time intervals and different concentrations were tried for each.

A laboratory method was developed whereby the efficiency of the fungicides can be tested using living plant materials in a test tube.

The tolerance of the spores of *C. thielavioides* to the fungicides tested was very close to the tolerance of Manetti cuttings. None of the 28 fungicides tested would kill all of the spores without seriously injuring the cuttings.

Since it was impossible to kill the spores of *C. thielavioides* under very favorable laboratory conditions it would seem impossible to find a chemical treatment that could be used on field-grown Manetti where the spores would be buried under earth and callus tissue.

Sanitary measures to prevent infection seem to be the best means of controlling this disease.

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