THE EFFECTS OF CERTAIN MERCURIAL FUNGICIDES ON GLADIOLUS PLANTS

by "

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THE EFFECTS OF CERTAIN MERCURIAL FUNGICIDES ON GLADIOLUS PLANTS

INTRODUCTION

Approximately 550 to 750 acres of gladiolus are grown in Oregon with an annual return of one to one and one-half million dollars from sale of corms and cut flowers. It has been estimated that losses of 10 to 20 per cent occur annually due to disease (53. p. 1). Corm rots caused by Sclerotinia gladioli Drayton and Fusarium oxysporum f. gladioli (Massey) Snyder and Hansen cause the heaviest losses in the gladiolus growing areas in Oregon. In trials conducted during the past 3 years it was found that Fusarium and Sclerotinia corm rots could be controlled quite efficiently under Oregon conditions by immersing the corms in a water suspension of New Improved Ceresan before planting. Because of reports of injury to gladiolus plants resulting from treatments with mercurial fungicides, it seemed desirable to investigate the effects of several mercurial fungicides on gladiolus plants. A fungicide, to be satisfactorily used on a commercial scale, must have fungicidal or fungistatic action toward the causal agent of the disease and must also be relatively non-toxic to the host plant involved. Otherwise benefits derived from control of a gladiolus disease could be partially offset by use of a fungicide that delayed flowering or reduced size of corms.

These investigations were conducted to determine the effects of pre-planting corm treatments with different mercurial fungicides on emergence, flowering, and size and number of corms produced. In addition, an attempt was made to correlate such observable effects with the amount of mercury present in corms produced.

LITERATURE REVIEW

Mercury Injury to Plants

In 1901 Dafert (13) reported that mercury caused injury to plants but it was not until 1934 that an extensive investigation was conducted to determine the extent of damage to plants due to the effects of mercury. In 1934 Zimmerman and Crocker reported that 65 of the 75 species of treated plants that they examined were injured by mercury or mercury compounds (54). Since their investigation there have been several reports concerning the effects of mercury on other plants such as <u>Lupinus albus</u> (31), Narcissus (34), tomatoes (14), corn (24) (37) (42), and other plants (6) (19) (38) (45) (47). Delay of germination, marring of the treated surface, deformation of parts of the plant and reduction of yield have been the usual injuries reported.

Injuries to gladiolus plants resulting from the use of mercurial fungicides in pre-storage or pre-planting treatments have been mentioned frequently in the literature but only Hawker (21) and Gould (17) have presented data substantiating their observations.

In 1928 Wedgworth (50) reported that soaking corms in mercuric chloride for 32 hours caused injury to corms with husks removed. When corms were immersed for 64

hours, the treatment resulted in severe injury to corms, poor growth of plants and small yield of corms and cormels. When husks were not removed, corms were not injured as much as with the same treatment when they were removed. Other workers (10) (40) also have reported mercuric chloride to be injurious to corms of some varieties. Martin (33) reported that immersing corms for 7 hours in mercuric chloride plus hydrochloric acid caused the formation of brown, sunken areas on corms and a delay of germination. A delay of flowering also has been reported (40) (52) when mercuric chloride treatments were used. When used as a pre-storage treatment, mercuric chloride delayed shoot emergence and flowering, depending on length of time between digging and treating (20). Greater delay occurred when this interval was short.

Pre-planting treatments with calochlor, a mixture of mercurous and mercuric chlorides, was reported by Miles (35) to delay germination of corms, retard growth of plants, reduce length of spikes and result in a reduction of corms harvested. Calochlor was used at the rate of 3 ounces in 5 gallons of water with both a 5-minute and a 30-minute immersion period. More injury occurred with the longer immersion period.

Treatments with fungicides containing ethyl mercury chloride were found by Tilford to result in a high percentage of corms being killed and a delay of several weeks in flowering (49). However no mention was made of type of treatment, length of treatment or concentration of fungicide.

Ceresan and Improved Ceresan were harmful as corm dips and Ceresan, when used in the form of dust, caused stunted foliage, poor flowers and low weight of corms (22). New Improved Ceresan also was reported to cause injury to gladiolus corms (11).

Immersion of corms for 7 hours in 1 per cent Semesan mixture was found to delay flowering (49) and a 2 per cent mixture was found to stunt plants and flowers (6).

Mersolite W (phenylmercuric acetate) was found by Gould to retard both emergence and flowering (17).

Spray, Puraturf 177, New Improved Ceresan and Ceresan M to determine their effectiveness in disease control and their effect on the gladiolus plant. He concluded that in general mercury compounds delayed flowering (18). Hawker reached the same conclusion following tests with mercuric chloride, mercuric chloride plus hydrochloric acid, hot and cold Aretan, Uspulun, and mercurous chloride. She also found that increasing the immersion period increased the delay of flowering (21).

Mercury Analysis

Numerous methods of analyzing various materials for mercury have been reported but they can be placed in several large groups without too much difficulty. The groups would include titrimetric, colorimetric, gravimetric, volumetric, spectroanalytic, and microchemical methods. Cucuel (12) presented a comprehensive review of methods used prior to 1933.

When determining the amount of mercury present in plant or animal material, a colorimetric or a spectro-analytic method is ordinarily used. A colorimetric method was chosen for this investigation since a Beckman DU Spectrophotometer was available. Dithizone (diphenylthiccarbazone) was the colorimetric reagent used although other materials have been used successfully (46). Dithizone is not a specific colorimetric reagent for mercury analysis but is also used for other metals. However by maintaining the pH at 1 or below and having chloride ions in a sample being analyzed for mercury, other metals, with the exception of the noble metals will not interfere with the determination of mercury according to Maren (32). Under the conditions of this investigation the noble metals were not believed to interfere.

Colorimetric methods using dithizone as the colorimetric reagent have been used in determining the presence of mercury in several plant materials (25) (26) (51) including gladiolus corms following an immersion of the corms in a solution containing mercuric chloride (36). Mercury has been found in various other plant materials using other methods of analysis or other colorimetric reagents (4) (34) (46) (54).

MATERIALS AND METHODS

Fungicides

Four fungicides were tested at a range of concentrations: New Improved Ceresan (5 per cent ethyl mercury phosphate) at 2, 4, and 8 pounds in 100 gallons of water; Ceresan M (7.7 per cent ethyl mercury p-toluene sulfon-anilide) at 2 and 4 pounds in 100 gallons of water; Puratized Agricultural Spray (5 per cent phenyl mercury triethanol ammonium lactate) at 0.1, 1.0 and 10.0 milliliters in a liter of water; and mercuric chloride at 1, 5, and 10 grams in a liter of water.

Corms

Size 4 corms of the variety Picardy were used in all experiments. The corms were obtained from L. E. Weeks gladiolus farm at Salem, Oregon.

Treatment

A fungicidal mixture was prepared by mixing a weighed amount of fungicide with a small amount of water to which the spreading agent, Triton 1956 B, had been added at the rate of $\frac{1}{2}$ pint in 100 gallons of water. The mixture was then diluted to the required concentration.

Corms to be treated were counted in lots which were placed in separate netted sacks and immersed in a

fungicidal mixture. Three replications of each treatment were used in all trials with corms in all replications being treated at the same time. When time was the variable factor, to determine the effect of length of treatment, all lots to be treated with any one fungicide were placed in that fungicide at the same time and as intervals of time elapsed 3 sacks of corms were removed. For example 3 sacks of corms were removed in 30 seconds, 3 more in 5 minutes and so on. The fungicidal mixture was stirred periodically throughout the immersion period to maximize coverage and in the case of New Improved Ceresan and Ceresan M to keep the fungicides in suspension. The corms were allowed to drain before planting.

Experimental Plots

Two experimental plots were located in ground beds in a greenhouse on the college campus where an attempt was made to determine the effect of concentration of a fungicide on gladiolus plants. In both of these experiments each replication was made up of 50 corms. For one experiment corms were immersed for 30 seconds in a fungicidal mixture and planted March 3 and for the other the corms were immersed for 15 minutes and planted March 7. Various concentrations of fungicides were used in each experiment.

Another experiment was conducted to determine the

effect of length of immersion period on gladiolus plants. Immersion periods of $\frac{1}{2}$, 5, 15 and 45 minutes were used. The corms were treated and planted on April 11 in an experimental plot located on the plant pathology farm at Oregon State College.

In the plots in the greenhouse the corms were planted 3 inches deep in rows 18 inches apart with an average of 7 corms per foot of row. In the plot in the field the corms were planted 6 inches deep in rows 30 inches apart with 7 corms per foot of row.

The plants were watered regularly and were dusted with 5 per cent dichlorodiphenyltrichloroethane for thrip control at approximately 10-day intervals. When the plants were about 3 to 4 inches tall, a side dressing of a 10-20-0 fertilizer preparation was applied at the rate of 500 pounds per acre.

The corms in the plots in the greenhouse were dug
August 1 and corms in the field were dug September 17.
Following digging, the corms were cured and cleaned.
Cleaning consisted of removing roots and old corms as well
as 1 or 2 outer husks. After the corms were cleaned,
counts were taken of the number of corms present and their
weight was determined.

All 3 plots were arranged in random block formation so that data obtained from them could be analyzed

made at both the 1 and 5 per cent significance levels.

Determination of the Effects of Mercurial Fungicides on Gladiolus Plants

Emergence

Counts of the number of plants that appeared above the soil were taken at various intervals after the first plants were seen.

Flowering

The number of plants in flower was counted at 2to 4-day intervals throughout the season. A plant was
counted and the spike removed when any bud on the spike
had begun to expand and the color of the petals was
visible. Since data were to be taken concerning the
effect on weight of corms produced, the spike was removed
in such a way that only part of the small upper-most leaf
on the stem of each plant was removed.

Corm Number

The effect of mercurial fungicides on number of corms produced was determined by counting the number of corms after harvesting and cleaning. As there was very little disease in the lots grown in the greenhouse, no count was taken of the number of healthy corms present.

Only the total number of corms was taken. However in the field experiment both the healthy and the total number of corms were taken.

Corm Weight

The effect on weight of corms was determined by weighing the corms after they were harvested and cleaned. Only total weight was obtained for corms from the green-house plots. Average weight of each corm was calculated from the total weight.

In the field experiment both the total weight of all corms and the total weight of healthy corms were taken and the average weight of each corm was calculated.

Weight was taken as it was considered to be the most accurate indication of the size of corms.

Determination of Mercury in Corms Produced Method of Preparing Samples

Corms from the experiment located in the field and from one of the experiments located in the greenhouse were analyzed for the presence of mercury.

Ten corms were taken randomly from each of 2 replications in each treatment from the field experiment. The replications of each treatment in the greenhouse experiment were mixed and 2 lots of 10 corms each were taken randomly. A sample consisted of material from each of the 10 corms in a lot. Separate samples were obtained from the stelar and the cortical regions. Material was obtained from the cortical region by removing 2 flat pieces (2 millimeters thick) from the surface of a corm and cutting and removing a wedge-shaped piece of tissue from the exposed cut surface. The wedge-shaped piece of tissue from the cortical region did not include material from the stelar region but did include material from very near the stelar region. The material from the cortex of each corm weighed approximately 0.3 to 0.5 grams which made a complete sample weight from 10 corms of approximately 3 to 5 grams.

Samples from the stelar region were obtained by removing both the upper and lower portions of the corm to provide a clean, cut surface on both the upper and lower ends of the stele. The center of the stele was removed with a cork borer. A quarter-inch cork borer was used in removing the center of the stele from corms from the field and a 3/16 inch cork borer was used for the corms from the greenhouse. The different sized cork borers were necessary because the corms from the greenhouse were much smaller than those from the field. A sample consisted of tissues from the steles of 10 corms and weighed approximately 3 grams when obtained from the field experiment and

12 grams when obtained from the greenhouse experiment.

Method of Digesting Corm Samples

A sample of either cortical or stelar material was weighed and placed in a 250 milliliter Erlenmeyer flask with a standard taper ground glass opening to fit a 20inch reflux condenser. Forty milliliters of concentrated sulfuric acid, containing approximately 8 per cent fuming sulfuric acid, were added to the material in the flask. To prevent bumping a curved stirring rod was extended about 12 inch up the inside of the reflux condenser fitted into the ground glass opening of the Erlenmeyer flask. A small beaker was placed over the upper opening of the reflux condenser to prevent contamination with laboratory dust. The mixture was heated on a hot plate for approximately 2 hours after which time about 12 milliliters of 30 per cent hydrogen peroxide were added through the top of the reflux condenser with an eye dropper. Additional hydrogen peroxide was added at intervals until a clear solution was obtained. Approximately 4 to 5 milliliters of hydrogen peroxide were added in all. Excess hydrogen peroxide was removed by heating for approximately 10 minutes after the solution became clear. The solution was then cooled, diluted with distilled water to approximately 200 milliliters and placed in storage until several samples had

been digested, so that a large number of samples could be analyzed for mercury at the same time.

Method of Analyzing Digest for Mercury

A method basically similar to the method suggested by Maren (32) for determining the amount of mercury present in organic material was used.

In making an analysis for mercury the contents of a digestion flask were placed in a separatory funnel to which 25 milliliters of 0.25 N hydrochloric acid and 2 milliliters of 20 per cent hydroxylamine hydrochloride had previously been added. To the mixture 22 milliliters of 30 per cent acetic acid solution were added to allow the analysis to be completed in light as no dark room was available in the laboratory (27). The mixture was then shaken before the dithizone solution was added in order to reduce any hydrogen peroxide that might still be present in the digest. This was done because dithizone in solution is very sensitive to oxidizing agents. Ten milliliters of a solution of dithizone in chloroform (10 milligrams of diphenylthiocarbazone dissolved in a liter of chloroform) were then added to the solution in the separatory funnel. The mixture was shaken 100 times and the chloroform layer collected. The per cent of light transmission of the chloroform layer which contained the

dithizone and a mercury dithizonate complex was determined with a Beckman DU Spectrophotometer. The per cent of light transmitted was determined at the 490 millimicron wavelength. At this wavelength dithizone transmits light quite freely while the mercury dithizonate complex transmits light less readily than at other wavelengths.

The transmission reading obtained from the unknown was compared to a calibrated curve plotted from transmission readings obtained with known quantities of mercury treated in the same manner as the unknowns. The amount of mercury in the digest was determined from this calibrated curve.

To determine if mercury was lost in the digestion process, known quantities of mercury were added to mercury-free gladiolus tissues. The gladiolus tissues were digested in the usual way and the contents of the digestion flask were analyzed for mercury as with the unknowns.

Approximately 12 per cent of the mercury added was lost during the digestion of the gladiolus corm tissues.

Effect of Concentration of Fungicide

Certain concentrations of all the fungicides tested (New Improved Ceresan, Ceresan M, Puratized Agricultural Spray and mercuric chloride) significantly delayed emergence when corms were immersed in the fungicidal solution or suspension for 30 seconds (Table 1). When corms were immersed for 15 minutes, all concentrations of each fungicide delayed emergence significantly (Table 2). In general greater delays occurred when higher concentrations of fungicides were used. The greater delays were particularly striking when a 15-minute immersion period was used. Total emergence was reduced significantly by immersing corms for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water. No other treatment reduced total emergence to such an extent.

The highest concentration of New Improved Ceresan (8 pounds in 100 gallons of water) delayed emergence significantly when corms were immersed in the fungicidal mixture for 30 seconds (Table 1). When corms were immersed for 15 minutes (Table 2), emergence was delayed significantly by all concentrations used. However, only the highest concentration for a 15-minute immersion period reduced total emergence significantly.

Emergence was delayed significantly by all concentrations of mercuric chloride when either a 30-second (Table 1) or a 15-minute (Table 2) immersion period was used. At the end of the season, however, there was no significant reduction of emergence when any of the mercuric chloride treatments were used.

A significant delay of emergence occurred when corms were immersed for 30 seconds in the higher concentrations (1.0 and 10.0 milliliters in a liter of water) of Puratized Agricultural Spray (Table 1). However there was no significant reduction of total emergence with Puratized Agricultural Spray.

Ceresan M was used instead of Puratized Agricultural Spray when corms were immersed for 15 minutes (Table 2). Both 2 and 4 pounds of Ceresan M in 100 gallons of water delayed emergence significantly, but neither concentration reduced total emergence significantly.

In general, higher concentrations of the fungicides delayed emergence to a greater extent than lower concentrations but the increased delay was not always significant. Figures 1 and 2 show graphically the effect on emergence of increasing the concentration of a fungicide. A comparison of the 2 figures reveals a greater delay when a 15-minute (Fig. 2) immersion period was used than when a 30-second (Fig. 1) immersion period was used.

Effect of Length of Immersion

In the experiment conducted in the field to determine the effect of length of immersion period on emergence, only the lowest concentration of each fungicide was used. New Improved Ceresan (2 pounds in 100 gallons of water), Puratized Agricultural Spray (0.1 milliliter in a liter of water) and mercuric chloride (1 gram in a liter of water) were the fungicides used. Immersion periods of 30 seconds, 5 minutes, 15 minutes and 45 minutes were used. Results of the experiment are shown in Table 3.

Immersing corms for a longer time in either New Improved Ceresan or mercuric chloride delayed emergence significantly as compared with a 30-second immersion period. However, the same effect was obtained by increasing the length of the water treatments.

Increasing the length of immersion period with Puratized Agricultural Spray did not bring about a significant delay of emergence nor reduce total emergence significantly.

Table 1. Effect of different concentrations of mercurial fungicides in 30-second pre-planting corm treatments on emergence of gladiolus plants.

		Per		of Plan		
Fungicide	Rate	25	31	34	43	69
New Improved Ceresan	2 lbs/100 gal water	60.0	88.0	96.7	97.3	98.0
	4 lbs/100 gal water	64.0	91.3	97.3	98.7	100.7
	8 lbs/100 gal water	54.0	85.3	96.0	98.7	99.3
Puratized Agricultural	0.1 ml/l water	58.0	91.3	96.7	99.3	100.0
Spray	1.0 ml/1 water	42.7	81.3	97.3	98.0	98.7
	10.0 ml/l water	41.3	84.7	94.7	97.3	98.7
Mercuric Chloride	1 gr/l water	53.3	87.3	93.3	98.7	100.0
	5 gr/l water	40.0	76.0	89.3	95.3	96.0
	10 gr/l water	45.3	89.3	96.7	98.0	97.3
Nater	<u> </u>	68.7	89.3	96.0	98.7	98.7
LSD at .05 LSD at .01		13.8				3.2

Table 2. Effect of different concentrations of mercurial fungicides in 15-minute pre-planting corm treatments on emergence of gladiolus plants.

		F	Per Cent Days	of Plant		d
Fungicide	Rate	22	28	31	40	66
New Improved Ceresan	2 lbs/100 gal water	35.3	72.7	84.7	96.7	99.3
	4 lbs/100 gal water	33.3	68.7	79.3	92.7	97.3
	8 lbs/100 gal water	13.3	44.0	58.0	76.0	85.3
Ceresan M	2 lbs/100 gal water	43.3	82.0	93.3	97.3	98.7
	4 lbs/100 gal water	31.3	68.0	82.0	92.7	97.3
Mercuric Chloride	1 gr/l water	44.0	83.3	96.7	98.7	98.7
	5 gr/l water	16.7	65.3	88.0	96.7	98.7
Untreated		65.3	92.7	98.0	98.0	98.7
LSD at .05 LSD at .01		16.0				6.0

Table 3. Effect of length of immersion in mercurial fungicides in pre-planting corm treatments on emergence of gladiolus plants.

	Length of		Cent of Plants Emo	
Fungicide	Immersion	28	43	92
New Improved Ceresan (2 lbs/100 gallons water)	(Min) 5 15 45	58.3 33.0 27.7 20.7	97.3 94.0 94.7 92.3	99 • 7 97 • 3 99 • 3 97 • 0
Puratized Agricultural Spray (0.1 ml/l water)	155 155 145	29.0 24.7 41.3	95.3 95.7 95.0 96.3	96.3 96.3 99.0 97.3
Mercuric Chloride (1 gr/l water)	15 15 45	50.3 24.3 34.0	96.3 97.7 97.0 96.3	99.0 98.3 97.7 98.0
Water	15 15 45	59.0 32.7 30.3 17.3	95.3 95.7 94.0 96.3	92.7 96.0 96.0 97.0
LSD at .05 LSD at .01		23.3 31.4		3.3

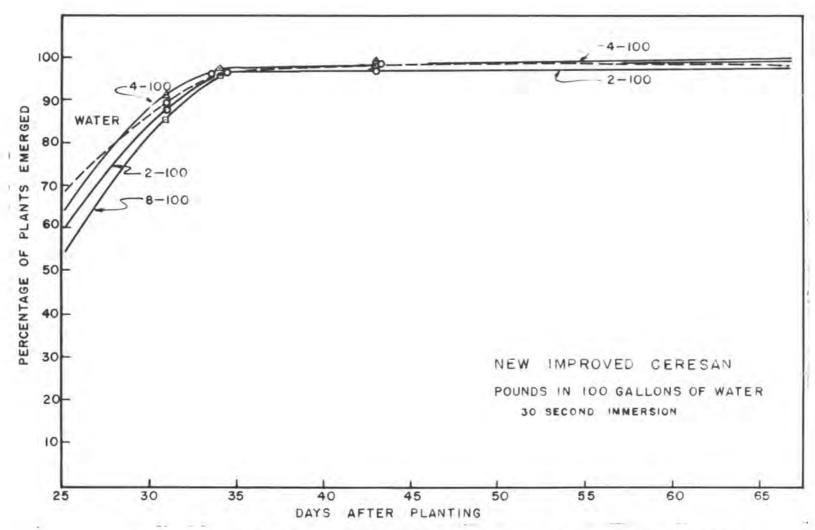


Fig. 1. Effect of different concentrations of New Improved Ceresan in 30-second pre-planting corm treatments on emergence of gladiolus plants.

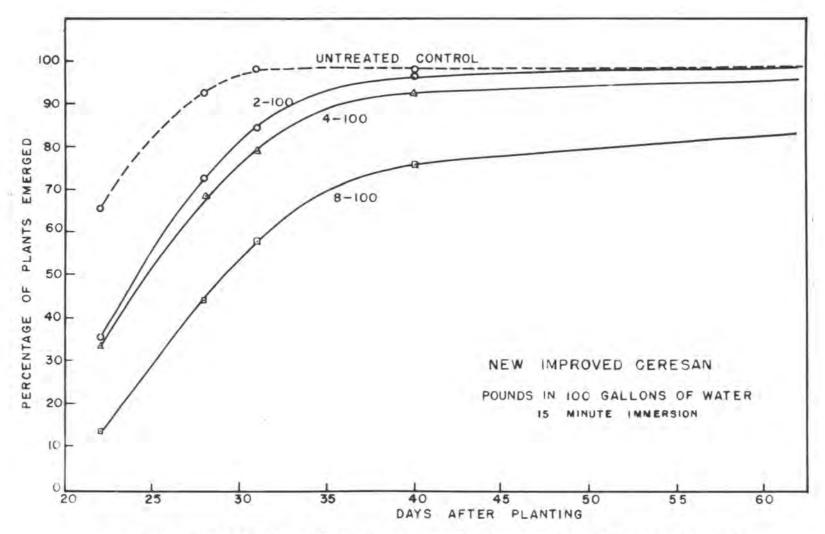


Fig. 2. Effect of different concentrations of New Improved Ceresan in 15-minute pre-planting corm treatments on emergence of gladiolus plants.

Effect of Concentration of Fungicide

Immersion of gladiolus corms for 30 seconds or for 15 minutes in some of the concentrations of all the fungicides tested significantly delayed flowering. In general greater delays occurred when higher concentrations of the fungicides were used. Total flowering was reduced significantly by immersing corms for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water.

Immersion of gladiolus corms for 30 seconds in 2, 4 or 8 pounds of New Improved Ceresan in 100 gallons of water delayed flowering as compared with a water treatment (Table 4). However, only 2 and 8 pounds in 100 gallons of water significantly delayed flowering. By the end of the season approximately the same percentage of plants had flowered from corms treated with the lowest concentration of New Improved Ceresan as from corms treated with water. Higher concentrations of New Improved Ceresan retarded flowering throughout the season but the retardation was not significant.

When a 15-minute immersion period was used, all concentrations of New Improved Ceresan delayed flowering early in the season, however, significant delays occurred only in lots treated in a suspension of 4 or 8 pounds in

100 gallons of water (Table 5). Flowering was retarded throughout the season by all concentrations of New Improved Ceresan but only the 8 pounds in 100 gallons of water resulted in a significantly lower per cent of flowering plants than the untreated.

Immersion of corms for 30 seconds in a solution of 1, 5 or 10 grams of mercuric chloride in a liter of water delayed flowering as compared with a water treatment, but a significant delay occurred only in the lots immersed in a solution of 5 grams in a liter of water (Table 4).

Approximately the same percentage of plants from corms treated with the lowest concentration of mercuric chloride and from corms treated with water had flowered by the end of the season. Corms treated with higher concentrations of mercuric chloride produced a lower percentage of flowering plants than those treated with water or the lowest concentration of mercuric chloride. However at the end of the season there was no significant reduction of flowering with any of the mercuric chloride treatments.

When corms were immersed for 15 minutes in solutions of 1 or 5 grams of mercuric chloride in a liter of water, flowering was delayed early in the season but only the more concentrated solution significantly delayed flowering (Table 5). At the end of the season there was no significant reduction of flowering with either treatment.

Results from immersing corms for 30 seconds in Puratized Agricultural Spray were similar to results obtained with New Improved Ceresan and mercuric chloride (Table 4). There was a significant delay of flowering with all concentrations (0.1, 1.0 and 10.0 milliliters in a liter of water) of the fungicide. However, by the end of the season there was no significant reduction of flowering with any of the Puratized Agricultural Spray treatments.

cultural Spray when a 15-minute immersion period was used. There was a delay of flowering when either 2 or 4 pounds in 100 gallons of water was used. A greater delay occurred when the higher concentration was used. However, neither delay was significant statistically (Table 5). By the end of the season the corms treated with the higher concentration produced a lower percentage of flowering spikes than the untreated but the difference was not significant. The corms treated with the lower concentration of Ceresan M produced a slightly higher percentage of flowering plants than the untreated.

Figures 3 and 4 show graphically the effect of higher concentrations of a fungicide on flowering. The results of treatments with New Improved Ceresan are presented in the graphs.

Effect of Length of Immersion

Immersing gladiolus corms for 5, 15, or 45 minutes in New Improved Ceresan caused a significant delay of flowering as compared with a 30-second treatment with the same fungicide (Table 6). There was no significant difference among any of the New Improved Ceresan treatments at the end of the season.

When corms were immersed in mercuric chloride for periods longer than 30 seconds, there was a significant delay of flowering as compared with the 30-second treatment. By the end of the season, however, there was no significant reduction of flowering with any of the mercuric chloride treatments.

Increasing the length of treatment with Puratized Agricultural Spray at the rate of 0.1 milliliter in a liter of water brought about no delay of flowering and the percentage of plants that had flowered by the end of the season was approximately the same for all immersion periods.

Immersion in water alone for varying periods of time had an effect on flowering similar to that observed following immersion in New Improved Ceresan or mercuric chloride. Flowering was significantly delayed by immersing corms for 5, 15, or 45 minutes as compared with a 30-second treatment. At the end of the season there was no significant difference among any of the water treatments.

No explanation is offered for the delay of flowering brought about by the water treatments.

Table 4. Effect of different concentrations of mercurial fungicides in 30-second pre-planting corm treatments on flowering of gladiolus plants.

			Per	Cent o				
Fungicide	Rate	118	121			132		169
New Improved	2 lbs/100 gal water	2.0	9.5	23.1	32.1	51.0	59.2	63.3
Ceresan	4 lbs/100 gal water	6.0	13.9	19.2	34.4	46.4	49.7	52.3
	8 lbs/100 gal water	4.0	11.4	18.1	30.2	40.3	48.3	55.7
Puratized Agri-	0.1 ml/l water	1.3	10.0	21.3	34.7	48.7	58.7	62.0
cultural Spray	1.0 ml/l water	0.7	8.1	18.2	27.0	37.8	44.6	48.6
	10.0 ml/1 water	1.4	5.4	10.1	20.3	33.1	42.6	52.0
Mercuric Chloride	1 gr/l water	4.0	15.3	20.7	34.7	46.0	56.0	62.0
	5 gr/l water	2.1	9.0	16.7	22.9	36.1	43.8	56.3
	10 gr/l water	2.7	13.7	21.9	36.3	43.8	49.3	52.7
Water		4.7	23.0	28.4	35.1	45.9	53.4	62.8
LSD at .05 LSD at .01			9.7			Kgan in a		18.2

Table 5. Effect of different concentrations of mercurial fungicides in 15-minute pre-planting corm treatments on flowering of gladiolus plants.

	Per Cent of Plants Flo Days After Plantin							
Fungicide	Rate	115	118		126	136	147	166
New Improved	2 lbs/100 gal water	6.0	10.1	16.8	20.8	21.5	27.5	32.2
Ceresan	4 lbs/100 gal water	3.4	5.5	11.6	17.1	21.2	27.4	34.2
	8 lbs/100 gal water	0.0	6.3	10.2	14.1	15.6	22.7	25.8
Ceresan M	2 lbs/100 gal water	6.8	14.2	19.6	23.6	29.7	35.8	43.2
	4 lbs/100 gal water	3.4	9.6	13.7	19.2	21.9	26.7	32.9
Mercuric Chloride	l gr/l water	2.0	12.2	20.9	24.3	29.1	30.4	36.5
	5 gr/l water	0.0	3.4	10.8	20.3	21.6	25.0	31.1
Untreated		7.4	18.2	27.7	30.4	32.4	36.5	41.2
LSD at .05 LSD at .01		e i		11.3				15.1

Table 6. Effect of length of immersion in mercurial fungicides in pre-planting corm treatments on flowering of gladiolus plants.

	Length	Per Cent of Plants Flowered Days After Planting							
Fungicide	Immersion	110	113	115		121	128	141	167
New Improved Ceresan (2 lbs/100 gal water)	(Min)	18.4 9.6 5.4 5.8	35.1 18.5 19.5 12.0	42.8 27.4 30.5 20.3	57.9 43.5 40.9 29.6	74.6 58.6 53.7 46.0	87.0 77.7 75.8 63.2	92.3 88.7 88.6 82.1	95.7 94.5 93.3 92.1
Puratized Agricultural Spray (0.1 ml/l water)	105 145	7.6 8.3 7.1 9.2	23.2 20.4 19.2 22.6	35.6 32.2 28.3 33.2	45.7 45.0 38.7 46.6	65.1 63.0 56.2 62.3	82.7 82.4 75.4 79.8	92.0 88.2 89.9 89.0	93.4 90.0 93.6 92.1
Mercuric Chloride (1 gr/l water)	10000 1400	16.2 3.1 5.8 1.7	31.3 15.3 19.1 7.1	43.1 28.5 32.1 17.0	54.5 41.7 45.7 22.8	70.7 61.0 66.2 38.1	84.8 86.4 87.4 74.5	92.9 94.9 95.2 96.9	94.9 96.3 97.3 99.0
Water	1555	19.8 8.7 9.7 5.2	39.9 24.0 20.5 16.6	45.7 33.0 32.6 28.5	56.1 44.4 41.0 43.5	66.5 63.2 57.6 63.7	74.1 80.9 80.2 85.0	81.7 91.0 88.5 92.7	84.5 92.7 92.9 93.8
LSD at .05 LSD at .01			12.1	distr.					5.8

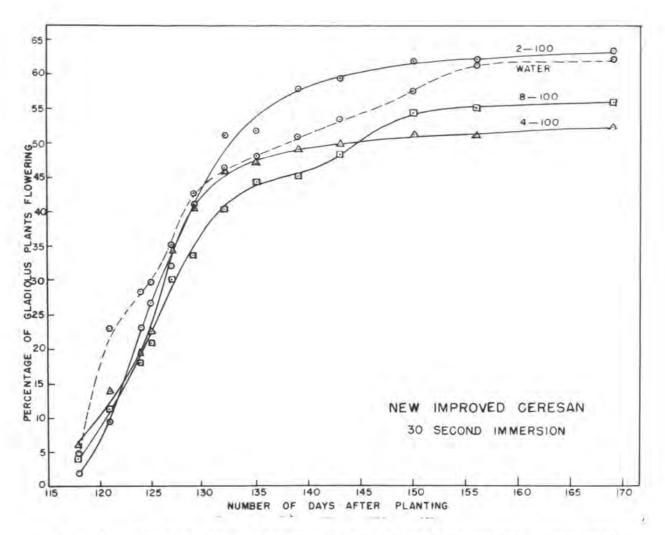


Fig. 3. Effect of different concentrations of New Improved Ceresan in 30-second pre-planting corm treatments on flowering of gladiolus plants.

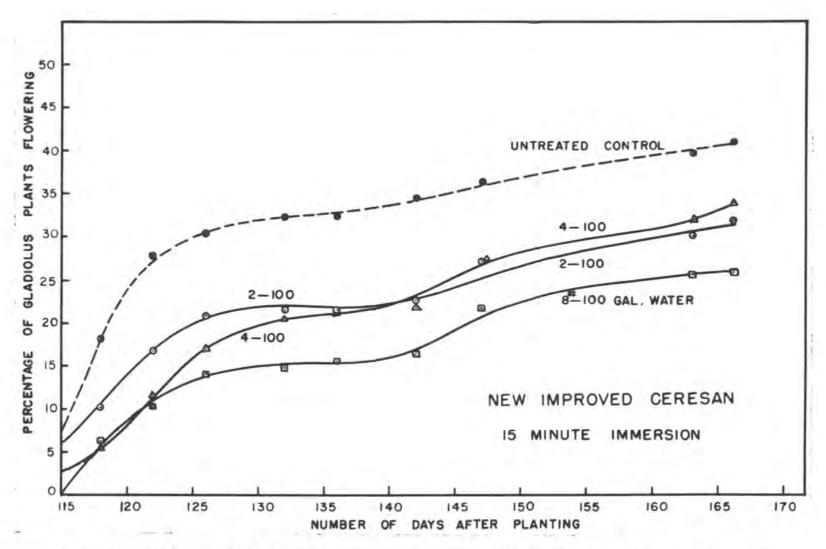


Fig. 4. Effect of different concentrations of New Improved Ceresan in 15minute pre-planting corm treatments on flowering of gladiolus plants.

EFFECT OF MERCURIAL FUNGICIDES ON SIZE OF CORMS PRODUCED

Effect of Concentration of Fungicide

The size of corms harvested at the end of the season was smaller in those lots that had received preplanting corm treatments with mercurial fungicides. The reduction of corm size was not as pronounced when treatments were 30 seconds long (Table 7) as when treatments were 15 minutes long (Table 8). With the shorter treatments, both mercuric chloride and New Improved Ceresan brought about a reduction of corm size when higher concentrations of the fungicides were used. However, none of the 30-second treatments reduced corm size significantly as compared with a water treatment.

Larger corms resulted from immersion for 15 minutes in the lowest concentrations of New Improved Ceresan and Ceresan M (2 pounds in 100 gallons of water) as compared with an untreated series. The larger corm size was not significant, however. These findings are similar to Hawker's observations with gladiolus (21). She found that larger corms were formed following a treatment with mercuric chloride. In this investigation higher concentrations of the fungicides significantly reduced corm size as compared with the lower concentration. The highest concentration of New Improved Ceresan (8 pounds in 100

gallons of water) and the higher concentrations of Ceresan M (4 pounds in 100 gallons of water) and mercuric chloride (5 grams in a liter of water) significantly reduced corm size as compared with the untreated.

Effect of Length of Immersion

In general longer treatments with mercurial fungicides resulted in the production of smaller corms but the reduction of size was not always significant (Table 9). Corm size was significantly reduced by 45-minute treatments with mercuric chloride, Puratized Agricultural Spray and water as compared with 30-second treatments. The 5- and 15-minute treatments also resulted in smaller corms.

Treatments with New Improved Ceresan did not produce as clear an effect on corm size as did treatments with the other mercurial fungicides or with water. For instance larger corms resulted from a 45-minute treatment with New Improved Ceresan as compared with a 15-minute treatment.

EFFECT OF MERCURIAL FUNGICIDES ON NUMBER OF CORMS PRODUCED

Effect of Concentration of Fungicide

Treatments with higher concentrations of the fungicides did not significantly affect the total number of corms produced with the exception of an immersion for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water (Table 8). With this treatment the number of corms produced was significantly reduced as compared with a 15-minute immersion in 2 pounds of New Improved Ceresan in 100 gallons of water. However, none of the treatments was significantly different from the water treated (Table 7) or untreated (Table 8). When a 15-minute immersion period was used, more corms were harvested from the fungicide-treated lots than from the untreated lots, but the difference was not significant.

Effect of Length of Immersion

The longer immersion periods in the fungicides did not significantly affect the total number of corms produced. However, in lots receiving water treatments, a significantly lower number of corms were harvested from those receiving a 30-second treatment than from those receiving longer water treatments.

New Improved Ceresan was superior to mercuric chloride and Puratized Agricultural Spray for controlling corm rots (Table 9). All treatments with New Improved Ceresan resulted in a high percentage of healthy corms whereas only the 15- and 45-minute treatments with mercuric chloride and Puratized Agricultural Spray resulted in a high percentage. A higher percentage of healthy corms was obtained also from a longer immersion in water.

Table 7. Effect of different concentrations of mercurial fungicides in 30-second preplanting corm treatments on number and size of resultant corms.

Fungicide	Rate	Number of Corms	Weight of Each Corm
New Improved Ceresan	2 lbs/100 gal water	48.7	(gr) 13.0
	4 lbs/100 gal water	49.3	11.8
	8 lbs/100 gal water	47.7	12.3
Puratized Agricultural Spray	0.1 ml/1 water	48.0	13.0
	1.0 ml/1 water	47.3	11.9
	10.0 ml/l water	48.3	13.9
Mercuric Chloride	1 gr/l water	48.7	15.1
	5 gr/l water	47.0	13.0
	10 gr/l water	48.3	12.7
Water		47.0	14.5
LSD at .05 LSD at .01		2.3	3.0

Table 8. Effect of different concentrations of mercurial fungicides in 15-minute preplanting corm treatments on number and size of resultant corms.

Fungicide	Rate	Number of Corms	Weight of Each Corm
New Improved Ceresan	2 lbs/100 gal water	47.7	(gr) 13.0
	4 lbs/100 gal water	47.7	11.8
	8 lbs/100 gal water	41.7	10.6
Ceresan M	2 lbs/100 gal water	47.3	13.2
	4 lbs/100 gal water	47.3	10.2
Mercuric Chloride	1 gr/l water	49.0	11.8
	5 gr/l water	48.0	11.1
Untreated		45.3	12.4
LSD at .05 LSD at .01		4.7	0.9

Table 9. Effect of length of immersion in mercurial fungicides in pre-planting corm treatments on number and size of resultant corms.

Fungicide	Length of Immersion	Total Number of Corms	Corms Healthy	Weight of Healthy Corm
New Improved Ceresan (21bs/100 gal water)	(Min.)	98.0	98.0	(oz) 2.07
	5	99.0	99.0	2.03
	15	98.3	99.4	1.81
	45	97.0	99.7	1.95
Puratized Agricultural	ם	90.0	84.1	2.29
Spray (0.1 ml/l water)	5	90.0	83.0	2.08
	15	95.7	93.0	1.90
	45	91.7	90.2	1.72
Mercuric Chloride	효	97.0	92.8	2.01
(1 gr/l water)	5	96.0	92.7	1.98
	15	98.0	98.7	1.86
	45	97.0	96.2	1.76
Water	효	83.3	78.4	2.24
	5	92.3	85.3	2.06
	15	90.0	90.0	2.16
	45	90.7	86.0	1.95
LSD at .05 LSD at .01		4.7		0.27

PRESENCE OF MERCURY IN GLADIOLUS CORMS

An attempt was made to correlate the degree of injury to the gladiolus plant with the amount of mercury present in the corm produced. To accomplish this corms from two of the experiments were analyzed for mercury. Corms from the experiment in which treatments consisted of immersing corms for 15 minutes in various concentrations of mercurial fungicides and corms from the experiment in which length of immersion period was varied while concentration of fungicide was constant were analyzed for mercury.

More mercury was present in corms from treatments with mercuric chloride than from other treatments. Due to the variability of the results, however, it is impossible to say definitely that mercury was present in corms treated with the other mercurial fungicides. The stelar region contained more mercury than the cortical region. More mercury was present also in corms from a 45-minute treatment than in corms from a 30-second treatment with mercuric chloride.

Due to the variability of the data obtained, only the above generalizations can be made. No generalization can be made concerning the correlation of the amount of mercury present in the corm with the degree of injury to the plant other than to mention that there did not appear to be any correlation.

It is impossible to explain the variability of the data. However, there are several places in the method used for analyzing the corms for mercury where variability might develop.

- 1. Sampling.
- 2. Removal of hydrogen peroxide. Hydrogen peroxide is a very strong oxidizing agent and would oxidize diphenylthiocarbazone if they were to come in contact. To remove hydrogen peroxide from the digestion flask, heat was applied for approximately 10 minutes after the contents of the digestion flask became clear. Also 2 milliliters of 20 per cent hydroxylamine hydrochloride were placed in the separatory funnel before the diluted contents of the digestion flask were added. The contents of the separatory funnel were shaken prior to adding dithizone solution in order for the hydroxylamine hydrochloride to come in contact with and reduce the hydrogen peroxide.

By previous experimentation it had been found that heating and the addition of hydroxylamine hydrochloride prevented oxidation of dithizone. However, if all the hydrogen peroxide was not removed, the transmission readings would be affected due to the oxidation of part of the colorimetric reagent.

3. Dilution of contents of digestion flask. It did not seem necessary at the time to dilute the contents

of the digestion flasks to equal volume with distilled water as the dithizone solution was added to the entire sample. The final volume, following dilution, did not vary to any great extent among the samples. But this is believed to be a source of variation.

4. Presence of a large amount of sulfuric acid in the separatory funnel when dithizone solution was added. All of the acid used in the digestion process was present in a dilute form when the dithizone solution was added. The presence of the large amount of sulfuric acid affected the transmission readings. But the calibration curve was based on readings obtained when known quantities of mercury were present in the same amount of diluted sulfuric acid.

If the investigation was to be conducted again, the contents of the digestion flasks would be brought to equal volume and an aliquot taken from each and analyzed. This would remove the effect of a large amount of sulfuric acid present in the separatory funnel when the dithizone solution was added.

DISCUSSION

Certain concentrations of all the fungicides tested, New Improved Ceresan, Ceresan M, Puratized Agricultural Spray and mercuric chloride were detrimental to the efficiency of the gladiolus plant. In general a longer immersion period or a higher concentration of a fungicide resulted in a greater delay of emergence, a greater delay of flowering, and a greater reduction of size and number of corms produced.

It is of interest that immersing corms for 30 seconds in a suspension of 2 pounds of New Improved Ceresan in 100 gallons of water, which is the treatment recommended for efficient disease control under Oregon conditions, did not result in any great harm to the gladiolus plant. There was some delay of emergence and flowering early in the season but by the end of the season, emergence and flowering were similar to those resulting from a water treatment. Smaller corms were obtained from the fungicidal treatment than from the water treatment but they were not significantly smaller. A greater number of corms was produced from the New Improved Ceresan treatment than from a water treatment and a higher percentage of healthy corms was obtained from the fungicidal treatment. Higher concentrations or longer immersions in New Improved Ceresan resulted in greater harm to the efficiency of the plant.

It is impossible to explain the effects of water on the gladiclus plant. A possible explanation may lie in the water used. Water from the city water line was used to dilute the fungicidal solutions or suspensions to the required concentrations. As the city water was chlorinated, there is a possibility that chlorine may have brought about the results obtained with the water treatments. However, there is no experimental evidence to support such a suggestion.

Despite the variability of the data obtained from analyzing for the presence of mercury in the corms produced, two interesting inferences can be drawn. More mercury was present in corms as a result of a treatment with an inorganic mercurial fungicide than with any of the organic mercurial fungicides. This indicates that a small molecule containing mercury was taken up more readily than a large molecule. Evidently mercury was taken up through the vascular system of the old corm into the vascular system of the new corm, as more mercury was present in the stelar region than in the cortical region of the new corm.

Even though the effects of mercurial fungicides on emergence, flowering, size of corms and number of corms produced were considered separately, it may be assumed that they are intimately related. Undoubtedly some of the delay of flowering resulted from a delay of emergence.

However, the investigation was not conducted in a manner to differentiate clearly between the effect on emergence and flowering. It is well-known that a given variety of gladiolus will flower after a fairly constant number of days have elapsed following emergence. The number of days between emergence and flowering can be varied somewhat by varying environmental conditions, but with a given variety of gladiolus the number of days cannot be varied to any great extent. Therefore, it seems correct to assume that a delay of emergence may have brought about a delay of flowering.

Part of the reduction of corm size may have been due to the effect on emergence and flowering, although the physiology of the gladiolus plant has not been studied sufficiently to assume too much. It is known that the corm does not enlarge to any great extent prior to flowering. Therefore, it is possible that a delay of flowering reduced the time for the corm to enlarge prior to harvesting.

There is sufficient evidence in the literature to indicate that the effect of mercury on a plant may possibly be due to a general slowing down of metabolism.

It is difficult to explain the toxic action of mercury on plant metabolism since there are very few papers pertaining to the biochemistry of the toxic action of mercury to higher plants. However numerous studies have been conducted using animals, yeast, and bacteria, as well as isolated enzymes.

Hellerman presented an account of the effect of mercury and other inhibitors on urease, arginase, cerebrosidase and other enzymes in a review article in 1937 (23). Therefore the literature prior to 1937 will not be emphasized other than to mention that mercury compounds have long been known to poison enzymes and that chemicals such as hydrogen sulphide and certain mercaptans could suppress the poisoning action.

In 1940 Fildes (16), following a study of the action of mercuric chloride on bacteria, ascribed the antibacterial action of inorganic mercurials to an interaction with sulfhydryl groups in an essential compound.

Cook and his co-workers studied the effect of germicidal action of an organic mercury compound, phenyl-mercuric nitrate, and found that it acted as a non-specific enzyme inhibitor of all the enzymes tested including cyto-chrome oxidase, succinoxidase, succinic dehydrogenase, lactic dehydrogenase, glucose dehydrogenase and catalase (7). Cytochrome oxidase activity has been suppressed also by phenylmercuric hydroxide and p-chloromercuribenzoate (29) (43) and succinic oxidase activity by the latter compound (39). Not all enzymes were inhibited to the same

extent however. For instance succinoxidase activity was inhibited by a lower concentration of germicide than succinic dehydrogenase activity (7).

The effect of phenylmercuric nitrate on cytochrome oxidase activity was prevented by sulfhydryl-containing compounds, cysteine and glutathione (9); effect on respiration of yeast by cysteine and homocysteine (8) (9); and effect on growth of bacteria by cysteine, glutathione and homocysteine (48). However, the effect of phenylmercuric nitrate was not reversed even by adding sulfhydryl-containing compounds in excess. Cystine and methionine did not suppress the toxic action of mercury. but this was probably because the sulfhydryl groups are covered in those amino acids (48). Amino acids without sulfhydryl groups did not protect cytochrome oxidase or yeast respiration against the action of phenylmercuric nitrate (9). These findings and others (1) (3) (5) (30) suggest that depression of enzyme activity may involve interaction of a mercurial compound with essential sulfhydryl groups in the protein of an enzyme.

Two of the above mentioned enzymes, cytochrome oxidase and catalase, have not been found dependent on sulfhydryl groups for their activity, however (2). Nor has reversal of the action of phenylmercuric nitrate on yeast respiration (9), cytochrome oxidase activity (9)

or bacterial growth (48) taken place when sulfhydrylcontaining compounds have been added after phenylmercuric nitrate has been allowed to act. Salle and Ginoza (41) showed that the amount of protection of Staphylococcus aureus from the effects of mercuric chloride paralleled the number of free amino groups present. Greater protection was obtained with amino acids or split products of proteins than with proteins. These latter findings would indicate that some other part of the protein molecule may also be involved in the action of mercury on enzymes. This led Seibert (43), following studies on the effect of phenylmercuric hydroxide and p-chloromercuribenzoate on cytochrome oxidase activity, to suggest that there may be a denaturation of the protein moiety. Slater (44) was more definite and suggested that there may be not only a combination of the mercurial inhibitor with the sulfhydryl groups of the dehydrogenase enzyme but also an effect on the protein particles of the enzyme preparation which would affect the accessibility of the dehydrogenase to cytochrome oxidase. Kreke et al (28) found this suggestion in harmony with their own observations, as the cytochrome oxidase system with ascorbic acid as a substrate was found to be about as sensitive to phenylmercuric nitrate, phenylmercuric hydroxide, and p-chloromercuribenzoic acid as the anaerobic succinic dehydrogenase system. They also

reported that mercury concentrations that had only slight effect on succinic dehydrogenase and cytochrome oxidase completely inhibited the succinic oxidase system.

At the present time there is no explanation for the complete mechanism by which mercury is toxic to living organisms. There is sufficient evidence to indicate that mercury compounds interact with the sulfhydryl groups of the enzyme proteins. However, some enzymes have not been proven dependent on sulfhydryl groups for their activity. There is evidence that mercury may affect other parts of the protein molecule as well as the sulfhydryl groups or may possibly bring about a change in the physical characteristics of the protein molecule of an enzyme.

SUMMARY

rots has been effected by treating corms with New Improved Ceresan (5 per cent ethyl mercury phosphate). However, mercurial fungicides have often been reported as causing various types of injury to gladiolus plants. Because of this, investigations were conducted to determine the effects of pre-planting corm treatments with several mercurial fungicides on emergence, flowering, and size and number of corms produced.

- 1. Emergence was delayed to a greater extent by higher concentrations of the fungicides and longer immersion periods. Immersion of corms for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water resulted in a significantly smaller stand.
- 2. Flowering was delayed to a greater extent by higher concentrations of the fungicides and longer immersion periods. Immersion of corms for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water resulted in a significant reduction of flowering throughout the season.
- 3. With the exception of treatments with Puratized Agricultural Spray, higher concentrations of the fungicides resulted in production of smaller corms. Longer treatments with the fungicides also resulted in smaller corms.

- 4. The only treatment to effect a reduction of number of corms produced was a treatment consisting of an immersion of corms for 15 minutes in 8 pounds of New Improved Ceresan in 100 gallons of water.
- 5. Treatments with New Improved Ceresan were more effective in controlling gladiolus corm diseases than treatments with other fungicides.
- 6. More mercury was present in corms treated with mercuric chloride than with other mercurial fungicides.

 The stelar region contained more mercury than the cortical region. More mercury was present in corms from longer treatments.
- 7. No correlation of the degree of injury to the gladiolus plant with the amount of mercury present in the corm could be made.
- 8. New Improved Ceresan, when used at the rate recommended for controlling Fusarium and Sclerotinia corm rots under Oregon conditions, did not retard the activities of the gladiolus plant to an extent which would prohibit its use as a fungicide.

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