SELECTION BY LABORATORY ASSAY OF FUNGICIDES FOR FIELD CONTROL OF *BOTRYTIS GLADIOLORUM* TIMMERMANS

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INTRODUCTION

Bioassay techniques have developed rapidly in the past decade. Evaluation of the innate fungicidal action of chemicals is possible by methods recently established (20, 21). There is need, however, for a simple and rapid method of laboratory assay that will serve as a reliable criterion of field performance of fungicides.

Need for such a method of bioassay is becoming more acute in Oregon for three reasons: (1) The rapid advent of numerous organic fungicides has made available many new materials, (2) the specificity or selective action of these materials against certain disease organisms makes it desirable to evaluate as many as possible against each disease, and (3) the extreme diversity of Oregon's agriculture results in an unusually large number of plant disease problems and magnifies the job of field testing.

Climatic conditions in Oregon are such that leaf-spot diseases of plants may become devastating. The leaf-spot of gladiolus (*Gladiolus primulus* Baker), caused by *Botrytis gladiolorum* Timmermans, is an example of such a disease. During periods favorable for development of the disease gladiolus leaves may be almost totally destroyed, flowers may be blighted, and corms infected leading to their breakdown in the soil or later in storage.

Botrytis leaf-spot of gladiolus is a disease well adapted to a study of bioassay as a means of selecting fungicides for field performance. The disease is one of economic importance for which an effective control program is needed. The degree of infection may be measured quantitatively, making possible comparison between laboratory assay and field performance. The data presented in this thesis are the results of trials carried on over a two-year period.

LITERATURE REVIEW

A Botrytis disease of gladiolus was first described by Sorauer (33) in 1898. Since that time at least three distinct species of Botrytis have been associated with diseases of gladiolus. These are Botrytis gladioli Kleb. (27), B. cinerea Pers. (12, 25), and B. gladiolorum Timmermans (34).

In most of the reports of Botrytis-induced gladiolus diseases no attempt was made to identify the causal fungus specifically. Many authors assumed the pathogen to be B. cinerea (12, 25) and did not make comparative studies of B. cinerea and the pathogen commonly associated with gladiolus diseases in the field.

Klebahn (27) in 1930 described B. gladioli Kleb., a pathogen which he believed morphologically different from B. cinerea. Since Klebahn did not publish a description of the disease he was working with, did not perform inoculation trials, and did not maintain a culture of the fungus that he described, this name is not considered valid.

In 1942 Timmermans (34) reported the Botrytis pathogen to be a new species distinct from B. cinerea. He described the causal organism and named it Botrytis gladiolorum Timmermans n. sp. The fungus described by Timmermans differed from B. cinerea culturally and morphologically. Botrytis gladiolorum sporulated sparsely in culture,

producing subglobose conidia averaging 15 x 10 μ , while B. cinerea sporulated abundantly on most culture media producing ovoid conidia 10.1 x 8.0 μ .

Peiris (29) made a thorough comparison of B. cinerea and B. gladiolorum. His findings substantiated the earlier report of Timmermans. In addition Peiris investigated the host range of B. gladiolorum. He observed that pathogenically B. gladiolorum stood in strong contrast to other isolates of Botrytis. It was the only species which actively invaded gladiolus tissues. Botrytis gladiolorum did not attack such hosts as turnip, swede and lettuce while isolates of B. cinerea caused active infection on these hosts. Isolates of Botrytis described by Dodge and Laskaris (7), Hawker (12), McClellan et al. (23), Moore (27), and isolates used in this study were morphologically, culturally, and pathogenically similar to B. gladiolorum.

The Botrytis disease of gladiolus was first reported in North America by Drayton (8) who observed the disease on corms shipped into Canada from Oregon. The disease now occurs in many of the gladiolus-growing areas and is most serious in areas of California, Oregon, and Washington where climatic conditions favor the pathogen. Outbreaks of Botrytis leaf-spot and corm rot disease have been reported from Michigan (28), Florida (5, 17), and from New York (7, 38). Development of the disease in these states is sporadic and depends on the occurrence of favorable climatic conditions.

In some areas in the United States growers have lost over 50 per cent of susceptible varieties in years favorable for the occurrence of the disease.

Botrytis gladiolorum causes characteristic symptoms on leaves and corms. Descriptions of the disease, including foliar and corm symptoms, have been made by Hawker (12), Moore (27), Timmermans (34), Wade (36), and more completely by Peiris (29). The pathogen is capable of attacking the plant at any stage of its life cycle and may cause a leaf-spot disease, a neck rot or collar rot, and a corm rot.

The leaf-spot phase may become so destructive as to kill the foliage completely. Usually Botrytis leaf-spot appears first as a large number of small, rounded, reddish-brown spots on the leaves. As the spots enlarge they develop into lesions with pale brown centers and a well-defined, dark margin. Small spots may coalesce to form large irregular lesions. Leaf lesions of this type may cover the entire width of the leaf blade and cause the death of that portion of the leaf above the lesion.

In the field conidia are abundantly produced on infected leaves, particularly if the climatic conditions are favorable at digging time. These conidia may serve for secondary infection of either leaves or corms. The conidia are easily dispersed and may be carried by wind to plants in other parts of the field or may fall to the ground and infect corms.

Moore (27) first recognized three forms of symptoms on the corms and described them in 1939. These are an arrested lesion, core rot, and a spongy rot.

Arrested lesions are sunken, rounded, and straw-colored in the center with a darker margin. They result when conditions become unfavorable for development of Botrytis rot and the necrotic spot ceases to spread and becomes dry.

The second type of rot is a core rot which may involve the whole of the central tissue of the corm. Corms are often seen in which the central portion has completely dropped out. The outer flesh is often unaffected. Sometimes the basal flesh may still be intact, but very soft to the touch. This can often be used as a means of detection of core rot.

The third type of corm rot is a spongy decay involving the entire corm. This type of rot usually starts with small olive-green water-soaked lesions which soon develop into a soft brown rot. Such rot has often been found associated with the core rot. It has been suggested (27) that the spongy brown decay originates in the central core and spreads to the surrounding flesh.

Under humid conditions masses of the fungus mycelia develop on the affected parts. This is especially true under moist storage and transit conditions where temperature is likely to be most favorable. The three forms of symptoms

that develop on the corms are definite phases of the same disease, and according to Peiris (29) the forms of corm rots are probably varietal responses to environmental conditions, particularly temperature and humidity.

Timmermans (34) reported that Botrytis rot was much more prevalent on corms dug late in the season. Both Hawker (12) and Wade (35) reported similar results on the time of digging and the percentage of corm rot. Corms dug in September, October and November showed 0.5, 27.1, and 21.7 per cent of rot respectively.

Corms may be naturally infected after lifting. Infection may occur either through the cut end of the flowering stalk or through the stem scars left at the top and bottom of the corm at cleaning. Irrespective of how infection takes place most of the fungus spores come from infected foliage and cause heavy corm rot in storage (23).

In 1949 McClellan, Baker, and Gould (23) made a thorough study of the effect of temperature and humidity on the development of the disease. Correlation was noted between cool wet weather and the prevalence of the disease. It was suggested that both the foliage phase and the corm rot phase could be predicted with the aid of weather records in a given area.

Temperature has a marked effect on corm infection. Widely varying optimum temperatures for corm infection have been reported. McClellan et al. (23) reported the optimum

temperature for inoculation of freshly-harvested corms to be 35° F. Peiris (29) reported the highest proportion of successful infection of corms at 59° F. In all cases very little corm rot occurred at temperatures above 68° F. even though the optimum temperature for growth of the fungus in culture is about 70° F.

This was explained by the fact that gladiolus corms readily form periderm at higher temperatures (3) and are more resistant to infection. At temperatures above 71.5° F. suberization took place rapidly and infection was prevented. Suberization was much delayed and infection occurred when corms were stored at low temperatures. Corms stored at 40° F. for 79 days had very little wound periderm and at 59.5° F. wound periderm did not form until after 22 days (3).

It has been suggested (29) that the optimum temperature for attack may vary slightly in different varieties according to their cork-forming capacities. It is probable that varietal susceptibility to storage rot is at least partly determined by the tendency of corms to form periderm. Lack of infection of properly-cured corms at high temperatures was assumed to be due to rapid suberization and wound periderm formation. In view of this fact storage rot is essentially a low-temperature disease (29). The Botrytis corm rot continues in storage at temperatures too low for other types of corm rots to develop (27).

Foliage infection took place at much higher temperatures (23). The optimum for infection was between 55° and 65° F.

The optimum temperature for growth of B. gladiolorum in culture was 20° to 22.5° C., on prune agar at pH 5.6 (34). The maximum temperature for growth was about 30° C. and the minimum below 3° C. No growth occurred at pH 7 and above. Mycelium of the fungus developed abundantly on infected corms held under moist conditions. It was white, woolly, often profuse, and fluffy in texture. The growth on potato-dextrose agar or other common artificial media was similar to growth on the host. Sclerotia were abundantly developed on corms and on relatively old artificial cultures (34). Microconidia were formed on most media and were globose, 2 μ in diameter. Macroconidia were freely produced on flowers in the field and were sparingly produced on artificial media. Production of conidia tended to decrease with subculturing. Abnormal conidia were often found in cultures which received suboptimal illumination. These were also found mixed with conidia of the ordinary type (29).

Control of the Botrytis leaf-spot phase is very important since the leaf spots may serve as a reservoir of inoculum for secondary infection of leaves and corms. Such infection may lead to losses through decreased yield and increased storage rot of corms.

Many measures for control have been suggested for the

Botrytis corm rot (7, 27, 29, 35). Among these were early lifting, rapid drying at high temperatures, early cleaning, and field and storehouse sanitation. These methods reduced the amount of corm rot but were not completely satisfactory.

It has been reported that the Botrytis rot overwinters on corms in storage and can also be contracted from infested soil (27). Therefore, other control measures have been suggested such as discarding diseased stock (27), roguing (36), proper curing (28), and controlled storage temperatures (23). Only recently have some commercial growers obtained equipment and designed storage facilities so that corms can be dried rapidly after digging (23).

In the field of chemical control, spray treatments have been suggested by Gould (11) and Nelson (28), and more extensive reports have been by Hawker (12) and Magie (16, 17). Fungicides, such as Puratized Agricultural Spray, Dithane Z-78, and Fermate applied as sprays, and Pentachloronitrobenzene dust have been used. In addition, preplanting treatments of corms have been suggested by Magie (17), Nelson (28), Simmons (32), and Wade (36) as a means of controlling Botrytis.

Use of resistant varieties does not offer a satisfactory means of control since most of the desirable commercial varieties are susceptible (16, 27).

Laboratory Assay of Fungicides

Bioassay of fungicides had its beginning when Prévost (13) discovered the fungicidal property of copper in 1807. Following Prévost's observation little use was made of bioassay for over 100 years. Clark (4) and Wallace (37) renewed interest in bioassay just after the turn of the century. Their results were discouraging, however, since they were frequently not reproducible and did not correlate with field results.

The field of bioassay was reopened by workers (9, 18, 26) in England in 1935 when the thinking was shifted to standardization of testing. The significance of precision of mechanical application was demonstrated and it was shown that spraying distance and spraying time were as important as initial concentration.

During recent years methods of laboratory assay have been subjected to critical analysis which has resulted in marked improvement. Use of rigid controls and standardization of the methods of bioassay has greatly lessened biological errors, while recent developments in precision apparatus for applying sprays and dusts have materially reduced the mechanical variation. Bioassay developed so rapidly that in 1938 a committee to assist in standardizing the techniques of fungicidal assay was set up by the American Phytopathological Society (13, p.15).

Much progress has been made in bioassay of fungicides

in the last decade, largely because of demand for rapid assessment of new chemicals and use of statistical procedure (19). Precision apparatus for applying sprays and dusts has been developed in recent years. The settling tower, developed by McCallan and Wilcoxon (21) in 1940, and the horizontal sprayer, developed by Horsfall et al. (14) in the same year, are now used extensively in laboratories dealing with bioassay of fungicides.

The settling tower functions on the principle of filling the tower uniformly with the spray or dust and then allowing it to settle on a glass slide. The horizontal sprayer is a stationary apparatus in which the fungicide is sprayed horizontally through an atomizer nozzle onto a facing glass slide held a set distance away.

The principle methods of laboratory assay used today in this country are as follows:

Spore germination techniques

Slide-germination

Test-tube Dilution

Agar-plate Method

Mycelium-inhibition Technique

The slide-germination method (2) is standardized as one of the major methods of evaluating fungicides by the American Phytopathological Society.

The general description of the slide-germination technique is as follows. By precision technique, using

apparatus such as a settling tower or horizontal sprayer, fungicides are applied to chemically-clean glass slides. A series of dosages varying in geometric progression are used by regulating the deposition of the sprays. The slides are allowed to dry and then are placed in moist chambers. Fungus spores obtained under controlled conditions are suspended in distilled water and pipetted onto the sprayed or dusted slides. These are placed in moist chambers, sealed with water, and held at temperatures suitable for germination. The spores are examined for germination in the low power field of the microscope after a specified time, and a count of spore inhibition recorded.

One hundred potentially viable spores should be counted per deposit on concentrations of each compound. If the germ tube exceeds half the minor diameter of the spore it is arbitrarily defined as germinated (2).

The percentage of spore inhibition may then be plotted against the concentration of fungicide on logarithmic-probability paper (6). The best straight line is drawn through these points and the LD50 or LD95 obtained for comparing the relative merits of the different fungicides.

Peterson (30) suggested that the slide-germination method was faulty in that the drops containing the spores tend to flatten and spread, frequently flowing together or off the slide. It was reported that this could be corrected by gluing 12-millimeter glass circles to the slides with

petrolatum. These serve as standard surfaces onto which measured amounts of fungicides and spores are pipetted either separately or in combination.

It was suggested by McCallan, Wellman, and Wilcoxon (22) that water-soluble materials may be assayed by a test-tube dilution technique. This method is more simple and rapid than the slide-germination technique since no elaborate apparatus such as the horizontal sprayer or settling tower is required.

In this method a series of fungicide dilutions is prepared in test tubes. To a known quantity of chemical, usually two cubic centimeters, 0.5 cubic centimeter of spore suspension is added. The mixture is thoroughly stirred by blowing into it with a pipette before placing drops on the slides. Two individual drops of the resulting suspension are pipetted onto glass slides in moist chambers. These are left for a set time after which germination counts are made according to a standard method (1).

Gottlieb (10) reported a comparison of sensitivity of an agar-plate and test-tube dilution procedure for preliminary assay of fungicides. In the agar-plate method chemicals were combined with agar, plates poured, and spore germination trials made on the surface of the agar. This investigation revealed that both tests gave reproducible values for the toxicity of the chemicals. In experiments spores were used from the same collection and at the same

spore concentration in both tests. Eighty-five different organic compounds were tested against two fungi, Macrosporium sarcinaeforme and Sclerotinia fructicola. With the former organism 49.5 per cent of the compounds had the same toxic values by both methods. The agar-plate technique was more sensitive than the test-tube dilution method for 35.4 per cent of the compounds.

The addition of a spore stimulant was necessary in many cases to insure a high and relatively stable percentage of germination in the checks. As a source of nutrients in spore-germination tests, Wilcoxon and McCallan (39) have called attention to the stimulatory properties of tomato, orange, apple, or pear juice, and on aqueous extract of lily and gladiolus bulbs. Peterson (30) has suggested a nutrient solution made by extracting 0.1 gram of Difco potato-dextrose agar powder with 100 milliliters of water. It was found to produce a high percentage of germination.

The inhibition of mycelium as a means of laboratory assay has been used rather widely with certain fungi. It was shown by Falck in 1907 that the increase in diameter of a fungus colony was linear with time (13). Therefore, by comparing the growth of a fungus on treated agar with that on untreated agar the fungicidal value of a toxicant could be measured. This technique was developed and studied by many workers including Humphrey and Fleming (15), Richards (31), and Bateman (13).

The method consists of mixing the toxicant with agar just before it solidifies or by soaking mycelial mats in the toxicant and placing the mats on agar. Several chemicals and concentrations may be involved as well as different lengths of time for exposing the fungus to the chemical.

One may obtain a linear dosage-response curve from data on mycelial inhibition. The curve will provide the same type of information as similar curves for inhibition of spore germination (20).

METHODS

Isolation and Culture of the Pathogen

The cultures used in all of the following trials were isolated from naturally infected gladiolus leaves which were obtained from the Portland, Oregon area. Isolations were made by the following technique. Leaves with necrotic spots were surface sterilized in a 15 per cent concentration of commercial Clorox for one minute, then rinsed in sterile-distilled water. The leaves were placed on a sterilized glass slide and serial cross-sections were cut with a sterile razor blade. Five sections were placed on potato-dextrose agar in each petri dish. These were incubated at 20° to 25° C. Isolations were also made from infected corms by surface sterilizing as above and planting tissue on potato-dextrose agar. The isolates in all cases were identified microscopically and cultures were maintained on potato-dextrose agar slants.

Since the organism does not sporulate readily on ordinary media, it was necessary to grow the isolates on the special medium suggested by Pieris (29) in order to obtain spores for fungicidal assay.

The medium was as follows:

Glucose	5.0 grams
Peptone	6.0 grams
Potassium chloride	0.5 gram
Potassium phosphate K_2HPO_4	1.0 gram
Magnesium sulphate	0.5 gram

Sodium nitrate 2.0 grams
Make up to a liter with distilled water.

Temperatures between 20°-25° C., good light, and dehydration of the medium were necessary to stimulate sporulation. Under these conditions the culture sporulated sufficiently. If any of the factors above were lacking, sporulation was poor.

The organism was also grown on autoclaved gladiolus flowers where sporulation was abundant when the cultures were exposed to proper light conditions.

Laboratory Assay by Spore Germination Trials

Since screening large numbers of fungicides in field trials is impracticable, it was desirable to develop a rapid method of fungicidal assay with spores of B. gladiolorum.

The effect of a number of chemicals in the inhibition of spore germination was measured using an agar-plate technique. Spore germination trials were conducted on petri plates of two per cent agar and one per cent dextrose to which the required quantities of chemicals had been added to give concentrations of 1000, 100, 10, and 1 parts per million. Fifty milliliter lots of the medium were sterilized in 125-milliliter Erlenmeyer flasks, cooled to 45° C. and a quantity of chemical necessary to obtain the desired concentrations was then added to each flask. From each flask three petri plates were poured. Spores were taken for each test from ten-day-old cultures. Spore suspensions were made

by brushing the spores from inoculated flowers with a camel hair brush, placing them in sterile-distilled water and filtering them through sterilized cheesecloth. The desired concentration of spores was obtained by the use of a Neubauer haemocytometer. The suspension was adjusted to 50,000 spores per milliliter of liquid. Each plate was inoculated at four equidistant locations with .05 milliliter of the conidial suspension and the plates were incubated at 25° C. for 16 hours. A random count of 100 spores was then made on each plate and the per cent of spore inhibition recorded.

Laboratory Assay by Effect on Mycelial Growth

Tests of chemical inhibition of mycelial growth were made as follows. The organism was grown on plates of potato-dextrose agar at 20° C. until hyphae had almost touched the edge. From these plates disks of mycelium, eight millimeters in diameter, were cut with a sterilized cork borer. The disks were placed in 1000, 100, 10, and 1 parts per million concentrations of the chemical to be tested. After two, 24, and 48 hours three disks were removed from each concentration, washed in two changes of sterile-distilled water to remove chemicals, and placed on potato-dextrose agar slants to observe for growth.

Field Testing

Botrytis leaf-spot control experiments were conducted through two seasons. In 1949 a cooperative plot was established with Max Perrin at a location about ten miles west of Portland. In 1950 a test was conducted on the Lewis-Brown College Horticultural Farm four miles southeast of Corvallis.

In both trials a five-by-five Latin square field plot design was used. Each of the five replications included four chemical sprays and an untreated check. Each replication consisted of 30 feet of row in 1949 and 20 feet of row in 1950. Six bulbs were planted per foot of row. Chemical sprays were applied once a week starting approximately August 1 before Botrytis leaf-spot appeared. A three-gallon Hudson sprayer operated at a pressure of about 50 pounds was used for all spraying. Plantings were cared for by ordinary culture practices.

Sampling and Computing Disease Index

In both field tests the sampling for disease reading was made in the same way. Twenty-five plants were selected at random from each replication and cut at the ground level. The samples were taken to the laboratory and approximately 100 leaves were counted at random from each replication.

Five grades of disease were set up as follows:

0 = No Botrytis lesions.

1 = 0- 5 per cent of leaf area necrotic.

2 = 5- 25 per cent of leaf area necrotic.

3 = 25- 50 per cent of leaf area necrotic.

4 = 50-100 per cent of leaf area necrotic.

Each sample was graded into the above classes. Disease leaf samples graded into each of the above five classes are shown in Figure 1.

A disease index was then derived and placed on the basis of 100 per cent by application of the formula

$$\frac{X(0) + X(1) + X(2) + X(3) + X(4)}{4\,t}$$

Where X = Number of leaves in each class.

t = Total number of leaves counted.

4 = Number of highest disease class.

The accuracy of such a formula in evaluating levels of plant disease has been well established by McKinney (24).

Figure 1. Percentage classes used to determine a disease index of Botrytis leaf-spot of gladiolus.


0
0-5
5-25
25-50
50-100

ASSAY OF FUNGICIDES BY INHIBITION OF SPORE GERMINATION

Since Botrytis gladiolorum sporulates rapidly and heavily on infected plants, an abundant source of inoculum is available for infection of other plants. To prevent spread and total destruction of gladiolus foliage and flowers during periods favorable to the pathogen, it is necessary to control the disease by application of protectant or eradicant fungicides.

A large number of such materials is available at present. In fact, there are so many that field evaluation of the potentially effective fungicides is impractical. Therefore an accurate and rapid method of laboratory bioassay is needed by which the most promising fungicides can be selected in the laboratory for use in field control trials.

In selecting chemicals for bioassay, all of the fungicides that have been recommended for field control of B. gladiolorum were chosen. These, plus other promising fungicides, were screened for their effectiveness in preventing germination of spores of B. gladiolorum. A total of 16 chemicals were evaluated by spore germination trials. The chemicals used and per cent of inhibition of spore germination at various concentrations are shown in Table 1.

Ferric dimethyl dithiocarbamate 70% (Fermate), zinc ethylene bisdithiocarbamate 65% (Parzate), 2,3-dichloro--1,

TABLE 1. EFFECTIVENESS OF DIFFERENT CHEMICALS IN INHIBITING GERMINATION OF SPORES OF *BOTRYTIS GLADIOLORUM*

Chemicals Used	Per Cent Inhibition at Concentrations of (PPM) 1000	100	10	1	Check Germination
Malachite green	100.0	100.0	98.4	79.0	98.0
Disodium ethylene bisdithiocarbamate hexahydrate 25%	50.0	30.0	19.0	1.0	97.0
Zinc ethylene bisdithiocarbamate 65%	100.0	100.0	97.3	42.0	96.0
Ferric dimethyl dithiocarbamate 70%	100.0	100.0	98.7	26.2	97.0
Zinc dimethyldithiocarbamate 76%	100.0	100.0	65.0	6.0	95.5
2,3-dichloro--1,4-naphthoquinone	100.0	100.0	79.6	14.3	96.0
Tetrachloroparabenzoquinone	85.0	71.0	15.0	3.0	98.0
2,4,5-trichlorophenyl acetate 50% + 50% pyrax ABB	100.0	86.0	22.0	4.0	96.5
Phenyl mercury triethanol ammonium lactate 5%	100.0	100.0	50.0	4.0	98.0
Glyoxalidines mixed 56% + Lime	90.5	63.1	15.4	6.1	97.0
Tetra copper calcium oxychloride 45%	98.7	79.0	4.0	5.0	96.0
Tri-basic copper sulphate 26%	100.0	4.0	1.0	1.0	96.0
Copper carbonate 55%	100.0	9.0	5.0	1.0	95.5
Copper 8 quinolinolate 50%	99.0	97.0	96.0	45.0	95.5
Colloidal sulphur 30%	4.0	3.0	3.0	2.0	96.5
Bismuth subsalicylate	13.0	2.0	2.0	1.5	96.5

4-naphthoquinone (Phygon XL), phenyl mercury triethanol ammonium lactate 5% (Puratized Agricultural Spray), zinc dimethyldithiocarbamate 76% (Zerlate), and malachite green completely inhibited spore germination at concentrations of 100 parts per million. Inorganic copper and sulphur were relatively ineffective in inhibiting spore germination. One thousand parts per million of tetra copper calcium oxychloride and copper carbonate completely inhibited spore germination, but at a concentration of 100 parts per million inhibition was only four and nine per cent respectively. One thousand parts per million of colloidal sulphur inhibited only four per cent of spore germination.

The four most promising chemicals and the mixed glyoxalidines were tested further at concentrations of 50, 20, 10, and 1 parts per million. The glyoxalidine mixture was included because it has been recommended for control of similar diseases and because the addition of this compound to the group gave a wider range of bioassay results for comparison with field performance. Malachite green was not tested further since its undesirable green color makes it impractical for field use.

Ferric dimethyl dithiocarbamate was most effective in inhibiting spore germination followed by zinc ethylene bisdithiocarbamate, 2,3-dichloro--1,4-naphthoquinone, phenyl mercury triethanol ammonium lactate, and glyoxalidine mixture. By plotting the results listed in Table 2 on

TABLE 2. EFFECTIVENESS OF SELECTED CHEMICALS IN INHIBITING GERMINATION OF SPORES OF *BOTRYTIS GLADIOLORUM*

Chemicals Used	Per Cent Inhibition at Concentrations of (PPM) 50	20	10	1	Check Germination
Ferric dimethyl dithiocarbamate 70%	100.0	98.0	94.3	20.7	95.5
Zinc ethylene bisdithiocarbamate 65%	100.0	94.5	85.3	18.1	
2,3-dichloro--1,4-naphthoquinone	100.0	92.7	70.7	11.1	
Phenyl mercury triethanol ammonium lactate 5%	100.0	90.2	59.0	8.0	
Glyoxalidines mixed 56%	33.4	15.2	12.7	5.3	

logarithmic-probability paper* it was possible to obtain a linear dosage response curve. The percentage of spores failing to germinate was plotted on the vertical axis (probability scale) and the chemical concentrations were plotted on the horizontal axis (logarithmic scale). Figure 2.

From the dosage response curve LD50 and LD95 values were read directly by interpolation. The LD50 and LD95 are defined as the concentration at which 50 and 95 per cent of the spores fail to germinate. The LD50 and LD95 values and slope of the dosage response curve are shown in Table 3.

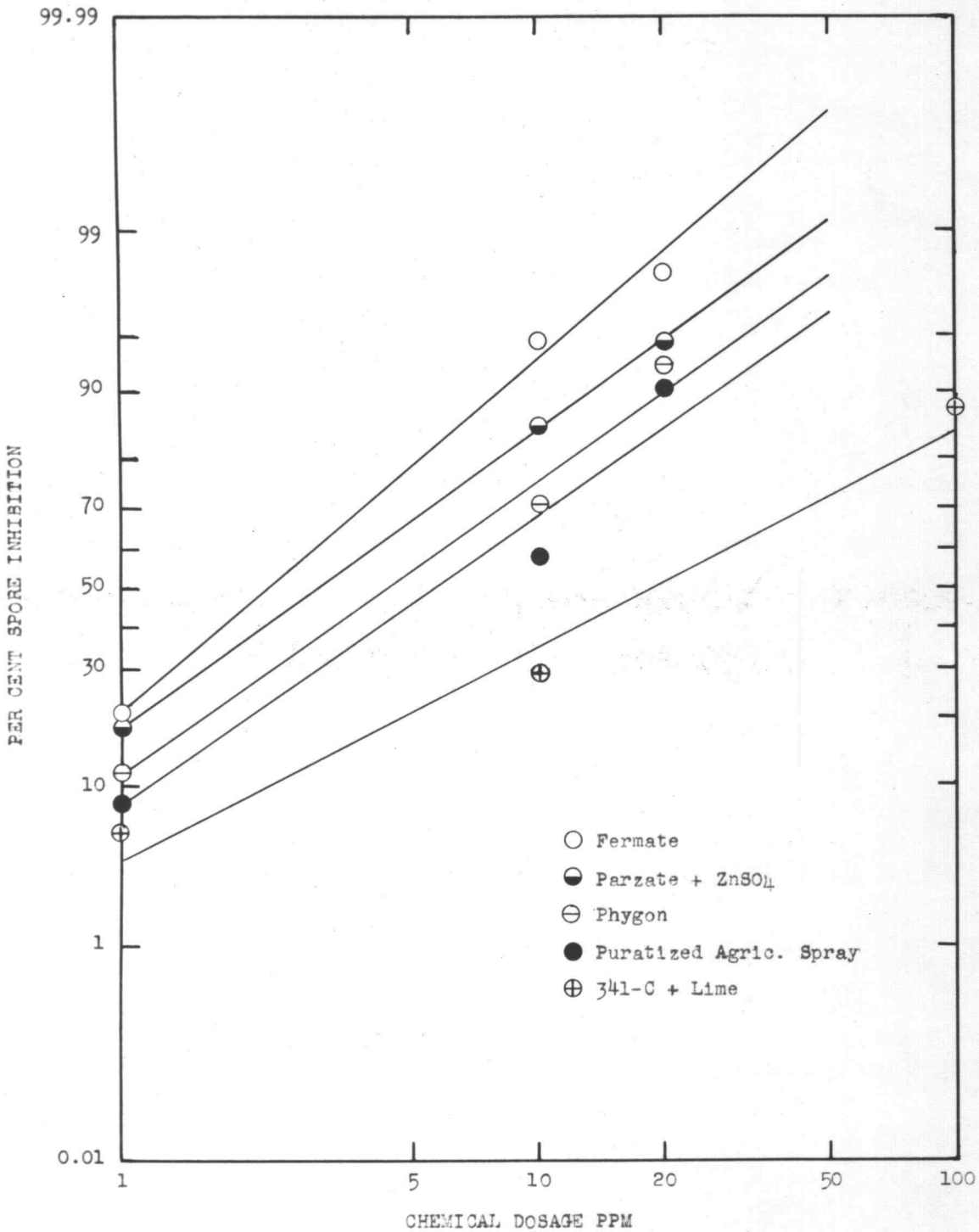
TABLE 3. LD50 AND LD95 VALUES AND SLOPE OF DOSAGE RESPONSE CURVE OF SELECTED CHEMICALS

Chemicals Used	LD50 PPM	LD95 PPM	Slope
Ferric dimethyl dithiocarbamate 70%	2.27	11.8	0.372
Zinc ethylene bisdithiocarbamate 65%	2.95	20.5	0.314
2,3-dichloro-- 1,4-naphthoquinone	4.35	31.4	0.295
Phenyl mercury triethanol ammonium lactate 5%	5.22	42.1	0.270
Glyoxalidines mixed 56%	18.5		0.197

Since the dosage response curves in Figure 2 do not cross, the LD50 and LD95 values indicate the same relative

* Logarithmic-probability Paper, Grid #3128, designed by Shipple and Fuller, Distributed by the Codex Book Company, Norwood, Massachusetts.

Figure 2. Toxicity curves for action of different chemicals on spores of *Botrytis gladiolorum*.


99.99
99
90
70
50
30
10
1
0.01
PER CENT SPORE INHIBITION
1
5
10
20
50
100
CHEMICAL DOSAGE PPM
Fermate
Parzate + ZnSO4
Phygon
Puratized Agric. Spray
341-C + Lime

effectiveness of the chemicals tested. Ferric dimethyl dithiocarbamate inhibited germination of 50 per cent and 95 per cent of the Botrytis spores at a lower concentration of chemical than did any of the other materials. Zinc ethylene bisdithiocarbamate, 2,3-dichloro--1,4-naphthoquinone, phenyl mercury triethanol ammonium lactate, and mixed glyoxalidines followed in the order listed. The range of dosages of chemicals required to kill 50 and 95 per cent of the spores was not great except for the mixed glyoxalidines. A much heavier concentration of mixed glyoxalidines was required for effective inhibition of spore germination than was needed for the first four materials listed.

ASSAY OF FUNGICIDES BY INHIBITION OF MYCELIAL GROWTH

The evaluation of fungicides by inhibition of mycelial growth has been of value, particularly to workers interested in wood preservatives. Since it was shown that rate of mycelial growth is directly proportional to time (13, p.38), the method has been used more extensively in the field of bioassay. The inhibition of mycelial growth is also useful in determining whether a chemical is fungicidal or fungistatic in action.

It was desirable to know if the materials used in the laboratory and field trials were fungicidal or fungistatic against mycelium of B. gladiolorum. A material is defined as fungicidal if it has the effect of killing the fungus. It is fungistatic if it is only inhibitory in action. A chemical that was fungicidal in action would be much more desirable for a foliage protectant under field conditions.

In mycelial inhibition trials, chemicals that had shown promising results in the laboratory and field were screened for their fungicidal or fungistatic action on mycelium of B. gladiolorum. The chemicals used and amount of growth at various concentrations are shown in Table 4.

Mycelial growth was completely inhibited after exposure for two, 24, and 48 hours to 1000 parts per million of malachite green, ferric dimethyl dithiocarbamate, zinc

TABLE 4. EFFECT OF CHEMICAL CONCENTRATION AND DURATION OF EXPOSURE ON THE RESPONSE OF MYCELIAL DISKS

Chemicals Used	Concentration	Growth After Exposure To Chemical For Hours		
		2	24	48
Malachite green	1000	-	-	-
	100	-	-	-
	10	-	-	-
	1	-	-	-
Ferric dimethyl dithiocarbamate 70%	1000	-	-	-
	100	+	+	-
	10	+	+	+
	1	+	+	+
Zinc ethylene bisdithiocarbamate 65%	1000	-	-	-
	100	+	+	+
	10	+	+	+
	1	+	+	+
2,3-dichloro--1, 4-naphthoquinone	1000	-	-	-
	100	+	-	-
	10	+	+	+
	1	+	+	+
Phenyl mercury triethanol ammonium lactate 5%	1000	-	-	-
	100	-	-	-
	10	+	+	+
	1	+	+	+
Glyoxalidines mixed 56% + Lime	1000	+	-	-
	100	+	-	-
	10	+	+	+
	1	+	+	+

ethylene bisdithiocarbamate, 2,3-dichloro--1,4-naphthoquinone, and phenyl mercury triethanol ammonium lactate. The mixed glyoxalidines also inhibited growth completely at 1000 parts per million but only after mycelium was exposed for at least 24 hours. Two factors were involved in inhibiting growth of the fungus. These were concentration of chemical and duration of exposure.

Since all mycelial disks used in these trials were thoroughly washed to remove any particles of chemical adhering to them, it is believed that failure of mycelium to grow after exposure to the chemicals tested is due to fungicidal action of the chemicals rather than to fungistatic action.

PERFORMANCE OF FUNGICIDES IN FIELD CONTROL

Following laboratory evaluation of fungicides for control of Botrytis leaf-spot the four materials which were most effective in inhibiting germination of spores of the fungus were further evaluated in field control trials in 1949. In 1950 trials glyoxalidine 341 was substituted for Puratized Agricultural Spray. It was desired not only to evaluate the performance of these materials against Botrytis leaf-spot, but also to compare laboratory and field performance of the fungicides to see if laboratory assay might be a reliable criterion of field performance.

In 1949 a trial was conducted cooperatively with Max Perrin near Beaverton, Oregon. A Latin square plot design with 30-foot replications was used in five rows of the gladiolus variety Pandora. At the end of the growing season 25 plants were selected at random from each replication of each treatment and approximately 100 leaves from these plants used to compute a disease index following the method described on page 20. The treatments used and the disease index of each replication of each treatment are shown in Table 5.

The trade names of the chemical compounds were used in preference to the chemical names since they are better adapted to use in tabulations. The chemicals and their trade names are as follows: Ferric dimethyl dithiocarbamate

TABLE 5. EFFECTIVENESS OF DIFFERENT FUNGICIDES IN CONTROLLING *BOTRYTIS GLADIOLORUM* AS MEASURED BY THE RELATIVE AMOUNT OF DISEASED LEAF AREA 1949

Replication and Treatment	DISEASE READING: No. of leaves in each disease class					Total		
	0	1	2	3	4	No. of Leaves	Disease Reading	Disease Index
1-1. Fermate	63	28	8	1	0	100	47	11.75
2. Parzate	58	38	2	2	0	100	48	12.00
3. Phygon XL	42	37	5	7	0	91	68	18.68
4. Puratized	0	63	27	9	0	99	144	36.36
5. Untreated Check	0	29	33	27	11	100	220	55.00
2-1. Fermate	55	41	3	1	0	100	50	12.50
2. Parzate	31	60	7	2	0	100	80	20.00
3. Phygon XL	4	66	16	11	5	102	151	37.09
4. Puratized	0	70	23	6	1	100	138	34.50
5. Untreated Check	0	60	30	9	1	100	151	37.75
3-1. Fermate	38	55	6	1	0	100	70	17.50
2. Parzate	16	68	15	1	0	100	101	25.25
3. Phygon XL	11	66	16	7	0	100	119	29.75
4. Puratized	7	68	18	7	1	101	129	31.93
5. Untreated Check	0	50	35	6	9	100	174	43.50
4-1. Fermate	51	46	3	1	0	101	55	13.61
2. Parzate	40	42	17	1	0	100	79	19.75
3. Phygon XL	12	52	25	10	1	100	136	34.00
4. Puratized	21	61	8	7	3	100	110	27.50
5. Untreated Check	0	48	26	19	8	101	189	46.78
5-1. Fermate	52	44	5	1	0	102	57	13.97
2. Parzate	50	39	10	2	0	101	65	16.08
3. Phygon XL	29	47	14	10	0	100	105	26.25
4. Puratized	3	58	21	16	2	100	156	39.00
5. Untreated Check	0	65	16	15	5	101	162	40.09

(Fermate)*, zinc ethylene bisdithiocarbamate (Parzate)*, 2,3-dichloro--1,4-naphthoquinone (Phygon XL), phenyl mercury triethanol ammonium lactate (Puratized Agricultural Spray), and glyoxalidine mixture (Carbide and Carbon's 341-C). A disease index summary and the average disease index for each treatment are shown in Table 6.

Similar trials were conducted at Corvallis in 1950 using the variety Picardy. Plot design and methods of sampling were as in 1949. The same chemical treatments were used with the exception of Puratized Agricultural Spray which was replaced by the glyoxalidine mixture. Results of the 1950 trials are shown in Tables 7 and 8.

In both the 1949 and 1950 trials weekly application of Fermate at the rate of two pounds per 100 gallons of water gave excellent control of Botrytis leaf-spot of gladiolus. The relative leaf-spot control with Fermate as compared to no treatment on gladiolus foliage is shown in Figure 3. All of the materials tested reduced the incidence of leaf-spot in the field. Following Fermate the most effective materials were Parzate, Phygon XL, Puratized Agricultural Spray, and 341-C in order of effectiveness.

Statistical analysis of the 1949 data showed that Fermate and Parzate were significantly better than Phygon and Puratized and that application of the latter two

* The American Phytopathological Society has given these two compounds the common names ferbam and zineb respectively.

TABLE 6. CONTROL OF BOTRYTIS LEAF-SPOT BY DIFFERENT FUNGICIDES AS INDICATED BY A COMPUTED DISEASE INDEX 1949

Treatment and Quantity Used in 100 Gallons of Water	DISEASE INDEX IN REPLICATION					Average Disease Index
	I	II	III	IV	V	
1. Fermate, 2 lbs.	11.75	12.50	17.50	13.61	13.97	13.867*
2. Parzate, 2 qts.	12.00	20.00	25.25	19.75	16.08	18.616*
3. Phygon XL, 1 lb.	18.68	37.09	29.75	34.00	26.25	29.154*
4. Puratized Agricultural Spray, 1 qt.	36.36	34.50	31.93	27.50	39.00	33.858*
5. Untreated Check	55.00	37.75	43.80	46.68	40.09	44.624

All spray mixtures contained Rohm and Haas Triton B1956 ½ pt./100 gals.

* Least significant difference at the one per cent level 9.52.

TABLE 7. EFFECTIVENESS OF DIFFERENT FUNGICIDES IN CONTROLLING BOTRYTIS GLADIOLORUM AS MEASURED BY THE RELATIVE AMOUNT OF DISEASED LEAF AREA 1950

Replication and Treatment	DISEASE READING: No. of leaves in each disease class					Total		
	0	1	2	3	4	No. of Leaves	Disease Reading	Disease Index
1-1. Fermate	8	84	8	0	0	100	100	25.00
2. Parzate	0	73	29	6	0	108	149	34.49
3. Phygon XL	0	18	56	26	5	105	228	54.24
4. 341-C	0	20	59	22	5	106	224	52.83
5. Untreated Check	0	17	38	33	12	100	240	60.00
2-1. Fermate	12	79	9	0	0	100	97	24.25
2. Parzate	1	53	40	7	0	101	154	38.12
3. Phygon XL	0	11	64	23	2	100	216	54.00
4. 341-C	0	19	51	28	6	104	229	55.05
5. Untreated Check	0	14	37	32	16	99	250	63.13
3-1. Fermate	25	65	12	1	0	103	92	22.33
2. Parzate	3	47	43	6	1	100	155	38.75
3. Phygon XL	0	14	55	27	2	98	213	54.33
4. 341-C	0	10	59	24	8	101	224	55.44
5. Untreated Check	0	12	40	35	13	100	249	60.25
4-1. Fermate	28	59	13	0	0	100	85	21.25
2. Parzate	1	39	56	4	0	100	163	40.75
3. Phygon XL	0	7	54	33	5	99	234	59.34
4. 341-C	0	6	53	34	8	101	246	60.89
5. Untreated Check	0	0	27	35	38	100	311	77.75
5-1. Fermate	19	78	3	0	0	100	84	21.00
2. Parzate	1	60	36	3	0	100	141	35.25
3. Phygon XL	0	16	53	27	5	101	223	55.19
4. 341-C	0	11	75	13	1	100	191	47.75
5. Untreated Check	0	16	51	25	8	100	225	56.25

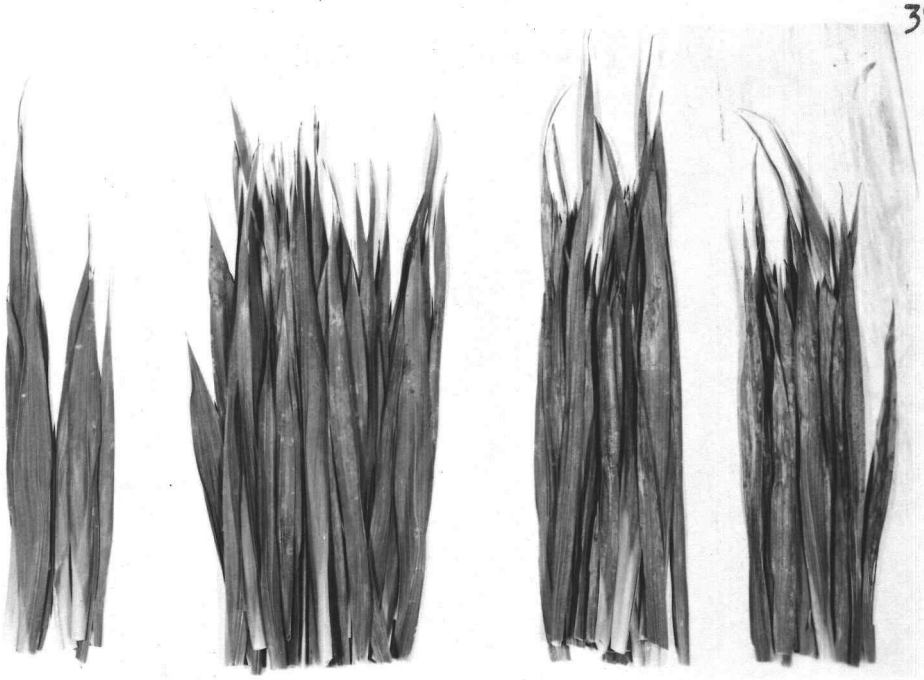
TABLE 8. CONTROL OF BOTRYTIS LEAF-SPOT BY DIFFERENT FUNGICIDES AS INDICATED BY A COMPUTED DISEASE INDEX 1950

Treatment and Quantity Used in 100 Gallons of Water	DISEASE INDEX IN REPLICATION					Average Disease Index
	I	II	III	IV	V	
1. Fermate, 2 lbs.	25.00	24.25	22.33	21.25	21.00	22.766*
2. Parzate, 2 qts.	34.49	38.12	38.75	40.75	35.25	37.472*
3. Phygon XL, 1 lb.	54.24	54.00	54.33	59.34	55.19	55.420*
4. 341-C, 1 qt.	52.83	55.05	55.44	60.89	47.75	54.392*
5. Untreated Check	60.00	63.13	60.25	70.75	56.25	62.076

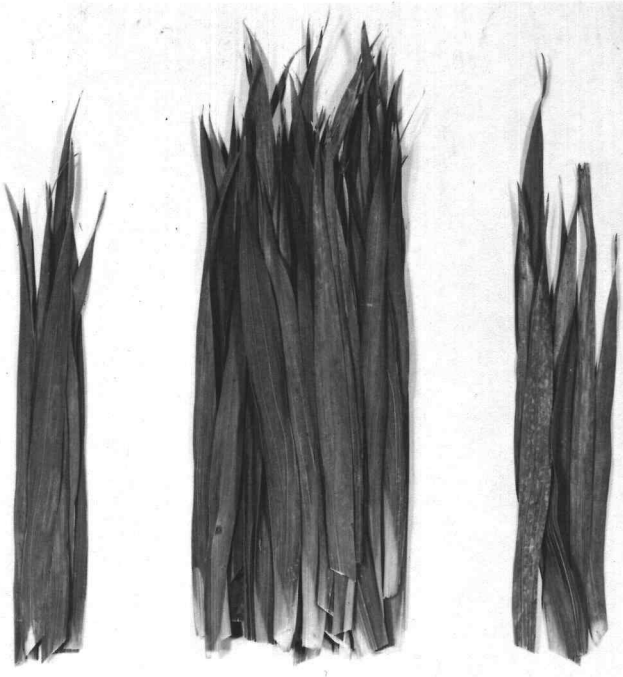
All spray mixtures contained Rohm and Haas Triton B1956 ½ pt./100 gals.

* Least significant difference at the one per cent level 4.32.

Figure 3. Gladiolus leaves graded into five percentage classes showing the effect of no treatment (above) as compared to Fermate spray (below).



0 0-5 5-25 25-50 50-100



0 0-5 5-25

materials resulted in significant reduction in disease as compared to the untreated checks. All tests for significance were made at the one per cent level. In 1950 Fermate again was strikingly superior to the other materials in the field. Analysis of the 1950 data showed Fermate to be significantly better than any of the other materials tested. Parzate was significantly better than Phygon XL and 341-C and the two latter materials caused a significant reduction in leaf-spot incidence as compared to the untreated check.

DISCUSSION

Ten years ago field evaluation of fungicidal chemicals was possible since the fungicide field was almost entirely confined to a few copper and sulphur fungicides for spraying and dusting, and to organic mercury compounds and formaldehyde for seed treatment.

Since 1940 a large number of new fungicidal chemicals have been made available for use in plant disease control. Many others are in developmental stages. The process of field evaluation of the fungicidal properties of these materials against the large number of plant diseases on Oregon's widely diversified crop plants is so costly in both time and materials as to be impractical. Yet, some form of evaluation is needed. Although Bordeaux mixture and a few of the older fungicides are generally effective, it is becoming more and more apparent that a certain specific chemical grouping is most effective against a specific disease. In other words, a fungicide may be highly effective against one disease and relatively ineffective against another. As a result of the advent of the large number of new fungicides on the market and their specificity for certain diseases, a rapid and accurate method of bioassay which will serve as a reliable criterion of field performance is needed.

The data presented in this thesis show a high degree of correlation between laboratory assay by spore germination

trials and field disease control of Botrytis gladiolorum. The laboratory assay failed to correlate with field trials in only one case. In laboratory assay Phygon XL was more effective in inhibiting spore germination than was 341-C, but the two were not significantly different in field control.

Definite proof has been offered that a rapid selection of the most promising fungicides for control of B. gladiolorum can be made by the agar-plate method of laboratory assay of fungicides. The method is simple, quantitative, and relatively rapid. It is preferred to the slide-germination and test-tube dilution methods for the following reasons.

In the agar-plate method spores are germinated in an environment that parallels natural conditions, there is no deficiency of oxygen and the water of the spore suspension diffuses into the agar after several hours leaving only spores on the surface. Therefore spore germination counts can be made without difficulty.

In the slide-germination method (20) spores may collect at the outer edge of the droplet where the concentration of the chemical may be higher, causing considerable variation in germination counts. Spore counts are more difficult to make in the slide-germination method because spores may be at different levels of focus in the drop.

A comparison of the agar-plate and test-tube dilution

methods has been made (10). The results of each test were reproducible and the agar-plate method was more sensitive in spore germination tests with 85 organic chemicals.

The method used for measuring field disease control of B. gladiolorum is accurate. Proof of this is shown by the results of field trials conducted through two seasons. These results were comparable when analyzed statistically. One fault of the method is a tendency to overemphasize the severity of disease, particularly in the lower classes. This is not a problem, however, to one who is familiar with the particular disease.

This study shows that laboratory assay offers a useful method of selection of fungicides for field trials. In both laboratory and field trials Fermate was most effective for the control of B. gladiolorum leaf-spot, followed by Parzate, Phygon XL, Puratized Agricultural Spray, and 341-C. Possibly one reason for the effectiveness of Fermate is the fact it is relatively insoluble in water, has good properties of adherence, and therefore is not readily leached away by rains.

The method of laboratory assay used in these trials proved to be a reliable criterion of field performance of the fungicides evaluated. No method of laboratory assay would be expected to be infallible and with trials with more fungicides exception would undoubtedly be encountered. However, the reproducible results obtained in two years' trials

show promise of great usefulness for this method of fungicide evaluation.

SUMMARY

A rapid method of laboratory assay based on inhibition of spore germination was employed to screen 16 fungicidal chemicals for use in field trials. The chemicals were screened by an agar-plate method.

The most effective chemicals were selected for further laboratory trials. Data on inhibition of spore germination by the chemicals were plotted on logarithmic-probability paper and a linear dosage response curve obtained. From the dosage response curve LD50 and LD95 values were obtainable directly by interpolation.

Five of the most promising fungicides were selected for field evaluation on the basis of their ability to inhibit spore germination. Ferric dimethyl dithiocarbamate, zinc ethylene bisdithiocarbamate, 2,3-dichloro--1,4-naphthoquinone, phenyl mercury triethanol ammonium lactate, and glyoxalidine mixture were used as spray treatments in field disease control.

Field trials were conducted through two seasons using a Latin square field plot design. At the end of each season disease readings were made by grading into five classes, leaf samples selected at random from each replication. On the basis of the number of leaves in each class a disease index was computed.

The laboratory assay by spore germination showed a

high degree of correlation with field trial results for the control of B. gladiolorum Timmermans. The relative effectiveness of the chemicals in preventing spore germination was comparable to their relative effectiveness in preventing development of gladiolus leaf-spot in the field. Fermate was most effective in laboratory trials and significantly superior to the other materials for leaf-spot control in field trials.

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