THE EFFECT OF SEED TREATMENT ON CONTROL OF DAMPING-OFF OF ORNAMENTALS; AND A STUDY OF A NEW FUNGAL PATHOGEN CAUSING DAMPING-OFF

Thesis for the Degree of Ph. D.

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This is to certify that the

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"The Effect of Seed Treatment on Control of Damping-Off of Ornamentals: and a Study of of a New Fungal Pathogen Causing Damping-Off."

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Ph.D. degree in Botany

William B. Drew
Major professor

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THE EFFECT OF SEED TREATMENT ON CONTROL OF DAMPING-OFF OF ORNAMENTALS; AND A STUDY OF A NEW FUNGAL PATHOGEN CAUSING DAMPING-OFF

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AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Department of Botany and Plant Pathology

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A study was conducted in which seeds of fourteen ornamentals were treated with certain of ten fungicides in various combinations in a series of field and greenhouse trials. In one or more trials significantly better seedling emergence occurred when seed protectants were used on seeds of Aster, Bachelor Button, Calendula, Cosmos, Four O'Clock, Morning Glory, Nasturtium, Phlox, Stock, Sweet Pea, and Zinnia. Marigold was the only ornamental on which seed protectants failed to give some measure of protection against damping-off. Only limited protection was apparent on Larkspur and Dahlia seed. Post-emergence damping-off was not controlled by the seed protectants in these experiments.

An organism, with a pycnidial imperfect stage, was isolated from a diseased Phlox seedling. This organism, by virtue of its perfect stage, was classified in the Aspergillaceae as that family is presently constituted. This organism was shown to be pathogenic and caused damping-off. A new genus and species, Pycnidiophora dispersa, were proposed and described to embrace this organism. The life history and morphology were studied on corn meal agar and potato dextrose agar. Conidium and ascospore germination and hypha development were traced. Pycnidial development was found to be simple meristogenous and the cleistothecium was observed to arise in a similar manner from a few intercalary cells in a single hyphal strand. The thirty-two spored asci, which were scattered throughout the cleistothecial cavity, were produced by crozier formation.

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Grateful recognition is given to the Northrup King and Company, Seedsmen, Minneapolis, Minn. and the Ferry Morse Seed Company, Detroit, Michigan who donated the ornamental seeds used in the investigation.

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INTRODUCTION

The fungal organisms causing seed decay and damping-off of ornamentals are limiting factors in seedling production. Tilford (43) said, "Practically all flower seedlings which are started in plant beds or in flats are subject to damping-off." There are no available official estimates of the annual losses caused by this disease in Michigan, but Andrews[1], maintains that damping-off is a very serious disease of ornamentals in the state. In routine visits to growers throughout the state he observed losses as high as ninety percent of the seedlings in a planting. According to Haney[2], it is a common practice among ornamental growers to plant at least ten percent more seed than would be required if the seedlings remained healthy, with growers frequently planting twice the required seed in order to overcome these losses. Aside from the added cost of seed, this practice necessitates thinning if damping-off is not as serious as expected, and is of little value if the disease is more destructive than anticipated.

Damping-off pathogens attack any or all parts of the seed

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1. Personal communication from Dr. E. H. Andrews, Extension Pathologist, Michigan State College, East Lansing, Michigan.

2. Personal communication from Dr. J. D. Haney, Professor, Department of Horticulture, Michigan State College, East Lansing, Michigan.

and seedling. Two distinct types of injury are usually designated, pre-emergence and post-emergence damping-off. When a young plant is killed before it reaches the surface of the soil, it is called pre-emergence damping-off. In this case the embryo may sometimes be killed before the hypocotyl emerges from the seed coat. Post-emergence damping-off occurs after the seedling has emerged above ground. This type of injury is characterized by a water-soaked appearance of the stem at the ground line, usually before the leaves wilt, followed by the toppling over of the infected seedling. Infection of the stem usually occurs at or below the ground level, but if the root is attacked, the entire root may be rotted away. Sometimes such plants will produce adventitious roots from the hypocotyl above the infection and recover, but these plants often remain stunted. If the cotyledons are attacked, the seedling may be stunted and deformed. As the plant grows older susceptibility to the disease diminishes.

Disease-free soil, washed sand, sphagnum moss, soil fumigation, soil sterilization, and seed treatment are used in attempts to control damping-off. Treatment of vegetable seed has been practiced for several years, and more recently seedsmen have been treating ornamental seed before packeting. However, new seed-treatment fungicides dictate the need for more information in regard to their powers of control of pre-emergence and post-emergence damping-off.

The purpose of this paper is to present the results from a series of tests of various seed treatment fungicides for the control of damping-off of some ornamentals. The objective of these seed treatment experiments is the determination of the protective value of the fungicides on fourteen ornamental species. In the course of these investigations a new pathogen (Pycnidiophora dispersa) causing damping-off of ornamentals was discovered, and a morphological study and a description of this fungus is presented in a later section of the paper.

HISTORICAL BACKGROUND

Many workers have published on various phases of damping-off and seed treatment. However, most of the work published is on damping-off of cereals or vegetables. A comprehensive review of the status of seed treatment with special reference to cereals was published in 1936 by Leukel (28). In an experiment station bulletin by Kadow and Anderson (25) in 1937 on seed and soil treatment an extensive bibliography was included. The following year Horsfall (22) summarized some of the more important literature on damping-off. Leukel (29) reviewed the cereal seed treatment literature in 1948, and in the same year Walker (45) reviewed vegetable seed treatment. Recently Linnasalmi (30) has given "a comprehensive, fully tabulated account of the author's studies at the Department of Plant Pathology, Tikkurila, Finland, on the etiology, distribution, and economic importance of damping-off in vegetables and ornamentals grown under glass, supplemented by a survey of the literature listed in an eight page bibliography."[1]

Although the parasites reported as causing damping-off are limited in number, a considerable number of such parasitic

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1. Since this article was unavailable the above quotation was taken from an abstract of the paper appearing in Rev. App. Mycology 32:412-413, 1953.

organisms are known. Species of Pythium, Rhizoctonia, Fusarium, Alternaria, Botrytis, and Phytophthora are some of the organisms capable of causing damping-off. Of these, Pythium spp., Rhizoctonia solani Kuhn, and Fusarium spp. are the more important organisms associated with seed decay and damping-off of ornamentals (4,6,13,15,28,30,32,35,37,38,39,47). According to Hartley (20) these forms are at least better known, if not more destructive, as damping-off organisms than as parasites on older plants.

Since Hesse (2) in 1874 first described, named, and demonstrated pathogenicity of Pythium debaryanum, this species has been proven to cause damping-off of a great number of host plants. As a rule P. debaryanum pathogenicity tests have been performed with vegetable and other types of plants. Very recently Srivastaya (38,39) using Hollyhocks and the ornamental Saponaria, and Linnasalmi (30) using Stocks have proven by inoculation and reisolation experiments that P. debaryanum causes damping-off of ornamental seedlings.

Rhizoctonia solani is another important cause of damping-off. Stock, China Aster, Carnation, Hollyhock, Snapdragon, and Saponaria were proven susceptible to this pathogen (4,6, 13,30,37,39). Baker (3) studied seed transmission of R. solani and found mycelium of this organism in the seed of Zinnia elegans.

Seed treatment for the control of plant diseases has been practiced for about three centuries. However, early

practices were not scientific because the nature and causes of plant diseases were not understood. Practices such as sowing in the dark of the moon were of a superstitious nature. Other early practices used from time to time consisted of the application of lime, salt, salt-peter, and wood ashes to cereal seeds.

Copper sulfate was suggested as a seed treatment by Schulthess in 1761 (46). General usage of copper sulfate, however, did not come about until a century later. Prevost (36) in 1807 demonstrated that copper prevented the germination of spores of the bunt fungus. Modifications in the recommendations of its use appeared during the middle 1800's, the most important of which, according to Leukel (28), was the addition of lime water to prevent seed injury. The hot water treatment developed by Jensen (24) in 1887 is one of the treatments used to kill pathogens inside the seed. Walker (45) points out that Guether in Germany in 1895 was the first to advocate the treatment of grain seed with formaldehyde solution. Its use was also advocated about this time, as a soil treatment to prevent damping-off (8).

Methods had been worked out for the control of some seed-borne pathogens of cereals by 1900, but attention in the first decade of the 1900's shifted to other seed-borne diseases particularly of vegetables. Mercuric chloride and other materials were tried up to about 1914 but were not generally recommended. However, after 1912, organic mercury

compounds were found to be effective seed treatment materials for cereal disease control. This marks the beginning of the use of organic materials as seed treatment compounds.

Walker (45) states that seed treatment for protection against soil-borne organisms did not receive much attention until after 1925. It soon became apparent, moreover, that fungicides applied to the seed would not only kill or inhibit seed-borne parasites, but would also give some measure of protection against soil inhabiting fungi (1,10, 12,19,21). Soon seed treatment dusts came into prominence and met with immediate popularity. With the successes of the organic mercury compounds other materials were investigated. Of these cuprous oxide and zinc oxide were found to be effective in many cases as seed protectants, but tended to cause injury. Because of the shortage of copper and mercury during World War II, a search for some organic materials was stimulated. Spergon, Arasan, and Phygon are examples of organic seed treatment compounds which are widely used. More recently liquid mercuric compounds such as phenyl ammonium mercuric acetate have come into use.

Tilford (43) in 1931 reported that the most effective control of damping-off was accomplished by the proper handling of a correctly sterilized soil, but that growers would not or, because of lack of equipment, could not steam sterilize soil. This method is satisfactory if recontamination of the soil is prevented. Other methods of soil treatment

for control of damping-off have been tried from time to time with varying degrees of success. Tilford (43) secured excellent control of the disease on 22 species of flower seed when he mixed six percent formaldehyde dust with soil at a rate of 1½ ounces per square foot. Two years later Wilson and Tilford (47) again improved the seedling stands of several ornamental varieties by the use of formaldehyde dust. Some varieties were reported by these authors not to be benefited, whereas others were injured by this chemical. Doran (15), in 1938, dealt exclusively with soil treatment for the control of damping-off of ornamentals. In this investigation he found that the following materials were of value in certain cases: washed sand, calcium cyanamide, acetic acid, pyroligenous acid, copper-lime dust, ammonia, formic acid, formaldehyde, acetaldehyde, salicylic acid, and aluminum sulfate. Lammerts (27) using five percent ethyl mercury iodide obtained good control of both pre- and post-emergence damping-off when the chemical was applied to the soil at the rate of 1½ grams per square foot. Dimock (13) reported that Rhizoctonia damping-off of Stocks can be controlled by sterilization of the soil with steam or chloropicrin (10 cc./cu.ft.). Later Newhall and Lear (33) recommended the use of methyl bromide (11 cc./cu.ft.) or Dowfume G (50 cc./cu.ft.) as soil treatments for controlling damping-off. The most recent soil treatment material, 8-quinolinol and its benzoate and sulfate salts, has been shown by

Stoddard and Zentmyer (40) to control both pre- and post-emergence damping-off of several ornamental and vegetable varieties.

The utilization of different substrates for the control of damping-off has also been tried. Dunlap (17), in 1936, obtained satisfactory development, combined with a reduction in the incidence of Rhizoctonia and Pythium types of damping-off when a number of seedlings were grown in pure brown sea sand supplemented with a mineral nutrient solution. In 1941 Stoutmeyer et al. (41) showed that damping-off of several ornamentals was prevented by the use of shredded sphagnum as a medium for seed germination.

Chemicals applied as liquid drenches have been tested for control of post-emergence damping-off. Dimock (14), in 1951, recommended a Semesan (1-2 lbs./100 gal. water) drench to control Rhizoctonia solani infections in Stock seed beds. Stoddard and Zentmyer (40) state that 8-quinolinol materials were definitely useful for the control of post-emergence damping-off when applied in aqueous solution.

In case the damping-off pathogen is seed-borne, particularly internally, more drastic measures may be necessary. Baker (3) found that the only successful treatment of seed of Zinnia infected with Rhizoctonia solani was hot water at 51.7° C (125° F) for 30 minutes, but that this treatment injured seed when more than one year old.

Because of the convenience of application and economy, a seed treatment fungicide capable of controlling both seed decay and seedling damping-off would be the most desirable approach to this disease problem. To study the control of damping-off, the injuriousness to seeds, and the stimulating effects of Red Copper Oxide, Horsfall et al. (23) in 1934 treated seed of 107 species and varieties of vegetable and ornamental plants with the dust. Many varieties responded favorably to this chemical, but there were some that showed a tendency toward injury, especially Legumes and Crucifers. In 1937, Taubenhaus and Burkett (42) also effectively controlled pre-emergence damping-off with Cuprocide (a cuprous oxide seed treatment). Person and Chilton (35) and Chilton et al. (9) reported that the best stands of ornamental seedlings were produced by treating the seed with Cuprocide and Yellow Copper Oxide. These investigators also found that improved stands were obtained with Vasco 4 and Spergon, while New Improved Ceresan, Ceresan, and New Improved Semesan Jr. frequently were toxic. Pre-emergence damping-off of Lilium regale was best controlled by seed treatment with Thiosan, Arasan, or Semesan, according to Doran (16), while Spergon, or Fermate similarly used gave inferior results as did several soil treatments. Recently, Linnasalmi (30) found that seed treatment would not control Rhizoctonia damping-off and only partial protection was given from Pythium debaryanum by excess dosages of Arasan, Tayssato, Dithane

Z-78, and Phygon. He reported that seed treatment could not be recommended for damping-off control in Finland and suggested that the use of a combination of physical and chemical disinfection methods would be more likely to prove successful.

MATERIALS AND METHODS

A series of seed treatment trials was conducted in the field and greenhouse from 1951 to 1953 for the control of damping-off. In these trials 14 different ornamentals were treated with various seed treatment chemicals (Table 1).

No attempt has been made in this investigation to evaluate the influence of environmental conditions on damping-off. Instead, by inoculation and maintenance of a high soil moisture, conditions were maintained which were favorable to the development of the disease. This was done in order to submit the various fungicide treatments to severe tests and to see whether they would still be of value under these extreme conditions.

The field tests were conducted on the Experimental Farms of Michigan State College. The plot of sandy loam with a pH of 7.2 was plowed and cultivated from a sodded condition to a garden consistency the first year and replowed and cultivated for the second year. Each year oat cultures of *Rhizoctonia solani* Kuhn[1] and *Pythium debaryanum* Hesse[2] were added and mixed into the soil during cultivation. The natural precipitation was augmented with a sprinkler system to maintain a high moisture level in the soil.

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1. The *Rhizoctonia solani* culture was obtained from Dr. William Klomparens.
2. The *Pythium debaryanum* culture was obtained from Dr. E. S. Beneke's culture collection.

TABLE 1

NAME, CHEMICAL COMPOSITION, AND SOURCE OF THE FUNGICIDES USED IN SEED TREATMENT OF ORNAMENTAL VARIETIES

Name	Chemical composition	Source
Arasan	Tetramethylthiuram disulphide (50%)*	E. I. duPont Company
Arasan SF	Tetramethylthiuram disulphide (75%)*	E. I. duPont Company
C & C 5400	Reaction product of dimethyldithiocarbamate and sulfur dichloride	Carbide and Carbon Chem. Co.
N. I. Ceresan	Ethyl mercury phosphate (5%)*	E. I. duPont Company
Orthocide 406	N-trichloromethylthio tetrahydrophthalimide (50%)*	Calif. Spray-Chem. Corp.
Orthocide 75	N-trichloromethylthio tetrahydrophthalimide (75%)*	Calif. Spray-Chem. Corp.
Phygon	2,3-dichloro-1, 4-naphthoquinone	U. S. Rubber Co.
Red Copper Oxide	Copper oxide (Red)	Rohm and Haas
Spergon	Tetrachloro-p-benzoquinone (96%)*	U. S. Rubber Co.
Vancide 51	Sodium dimethyl dithio carbamate - sodium 2-mercapto benzothiozate (30%)*	R. T. Vanderbilt Co.

*Percent active ingredient

The greenhouse trials were conducted in the Science Greenhouses of Michigan State College. The soil was prepared by mixing a rich loam with sand at a 1:1 ratio to which a small portion of well-decayed peat was added. This mixture was approximately pH 7. Oat cultures of Rhizoctonia solani and Pythium debaryanum were added to the soil at the time of preparation to augment the fungi naturally present. Such a soil mixture was used for two to three successive plantings. Afterward, about half of this soil was diluted with a new mixture, prepared as above, and replanted.

Regulation greenhouse flats were used for the plantings; light conditions were those normally found in a greenhouse for the time of year; an average temperature of 65° to 67° F was maintained; and the soil moisture was kept at a high level by daily watering.

For these tests seeds were obtained from commercial seedsmen with some seeds already treated with the chemicals used by the seed industry. When the seeds were treated in the laboratory, all chemicals except Vancide 51 were applied as dusts. The dust and seeds were shaken together in flasks until the seeds were well-coated. In all cases the excess dust was removed after treatment. Just enough Vancide 51, which is a liquid material, was usually applied to the seed to moisten the seed surface, after which it was allowed to dry. A non-treated control was planted with each replication in every varietal test.

The plantings were randomized in both greenhouse and field trials. Each variety was maintained as a separate unit for randomization purposes. In the greenhouse each replication was planted in a single flat by dividing the flat into the required number of short rows (Plates I, II, III). In the field the rows were approximately four feet in length and a foot apart.

As soon as the seedlings appeared a daily count of the emerged and diseased seedlings was made for a period of four days. Subsequent counts were made every third day for three weeks beyond the time of emergence. As the counts were made the damped-off seedlings were removed. Some of these diseased seedlings were placed in petri dishes and taken to the laboratory for further study.

Statistical analysis of the data accumulated for each variety in each trial was carried out by analysis of variance.[1] For purposes of analysis, missing numbers were estimated by Baten's (5) procedure.

Field Trials

The first trial was conducted in the field during the summer of 1951. Seeds of nine pre-treated ornamental varieties were obtained from the Northrup King and Company. The

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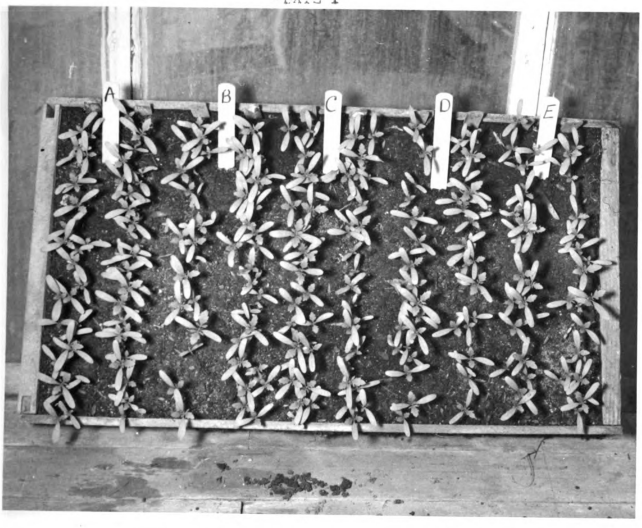
1. Assistance with the statistical methods was given by Dr. W. D. Baten, Statistician for The Experiment Station, Michigan State College.

PLATE I



Marigold seed treatment trial

A - non-treated
B - Spergon
C - Arasan
D - Phygon
E - C and C 5400

PLATE II

Calendula seed treatment trial

A - non-treated
B - Spergon
C - Arasan
D - Phygon

PLATE III

Aster seed treatment trial

A - non-treated
B - Spergon
C - Arasan
D - Phygon

materials used in these 1951 field trials are presented in Table 2. Table 3 summarizes the materials used in the 1952 field trials. The seeds were treated by the Northrup King and Company, with the exception of Sweet Pea which came untreated and was treated immediately before planting. The seeds were planted in late May and early June.

The third field trial was carried out during September of 1952. Bachelor Button and Zinnia (Giant of California) seeds from the same lots of seeds used in the earlier summer trial were used for this trial. The fungicides used and the amounts of each are given in Table 3. One hundred seeds per treatment per replication and four replications were planted for each ornamental.

Greenhouse Trials

Seeds from the 1951 summer test lots were used for the December 1951 greenhouse trial. The amounts of the various chemicals applied to the seed in ounces per 100 pounds are given in Table 2. One hundred seeds per treatment per replication of Aster, Calendula, and Marigold were planted for three replications. Two hundred seeds per treatment per replication of Larkspur were planted for three replications.

Table 4 summarizes the materials used in the 1952 greenhouse tests. Approximately the same amount of each fungicide was used in the two successive experiments with Sweet Pea and Stock. In the second experiment the lots of seed were

TABLE 2

NUMBER OF SEEDS PER REPLICATION AND AMOUNT OF FUNGICIDE APPLIED TO SEEDS IN THE SUMMER FIELD TRIALS OF 1951

Ornamental variety	Treatments and oz. of fungicide per 100 lbs. of seed				Number of seeds per treatment per replication	Number of replications
	Phygon	Arasan	Spergon	C & C 5400		
Aster, Crego	8	8	8	...	300	4
Calendula, Double Orange King	8	8	8	...	150	4
Cosmos, Sensation	4	4	4	...	200	4
Dahlia, Unwin's Dwarf Hybrid	...	4	4	...	200	4
Four O'Clock, Marvel of Peru	...	2	2	...	100	2
Larkspur, Giant Imperial	8	8	8	8	300	4
Marigold, Harmony Dwarf (French)	8	8	8	8	300	4
Morning Glory, Heavenly Blue	4	4	4	...	100	4
Nasturtium, Dwarf Choice	...	2	2	...	75	2

TABLE 3

NUMBER OF SEEDS PER REPLICATION AND AMOUNT OF FUNGICIDE APPLIED TO SEEDS IN THE SUMMER FIELD TRIALS OF 1952

Ornamental variety	Treatments and oz. of fungicide per 100 lbs. of seed						No. seed/ treatment/ replication	Number of replications
	Phygon	Arasan	Spergon	Arasan SF	Orthocide 75	C & C 5400		
Aster, American Beauty	8	8	...	5.3	5.3	...	100	4
Bachelor Button	8	8	8	5.3	5.3	...	150	4
Calendula, Double Orange King	8	8	8	5.3	5.3	8	100	4
Cosmos, Sensation	4	4	6	2.6	2.6	4	100	4
Dahlia, Unwin's Dwarf Hybrid	4	4	6	2.6	2.6	4	100	4
Four O'Clock, Marvel of Peru	2	2.5	4	...	2.0	2	50	4[a]
Larkspur, Double Mixed	8	8	12	...	5.3	8	150	4
Marigold, African	8	8	12	...	5.3	8	100	4
Morning Glory, Heavenly Blue	4	4	8	...	2.6	...	75	4
Nasturtium, Glor. Gleam Hybrid	2	2	4	...	2.0	...	25	4[b]
Zinnia, Giant of California	8	8	...	5.3	5.3	...	100	4
Sweet Pea[c]	8	...	...	4.0	...	4	100	4

a. Only enough seed in the Arasan treatment for three replications.

b. Only enough seed in the Arasan treatment for two replications of 25 seeds, one of 22 seeds; no fourth replication.

c. Sweet Pea seed also treated with N. I. Ceresan (2.5 oz./100 lbs.), Orthocide 406 (4 oz./100 lbs.), and Vancide 51 (6 oz./100 lbs.).

TABLE 4

NUMBER OF SEEDS PER REPLICATION AND AMOUNT OF FUNGICIDE APPLIED TO SEEDS IN THE WINTER GREENHOUSE TRIALS OF 1952

Ornamental variety	Month of trial	Treatments and oz. of fungicide per 100 lbs. of seed								No. seed/ treatment/ replication	Number of replications
		Phygon	Arasan	Spergon	C & C 5400	N. I. Ceresan	Red Copper Oxide	Orthocide 406	Vancide 51		
Calendula, Double Orange King	Jan.	ex[a]	ex	ex	ex	ex	..ex	...	X[b]	25	6
Stock	Jan.	ex	ex	ex	ex	ex	ex	...	X	25	8
Stock	Feb.	9.6	11.4	13.8	8.2	20.0	10.2	16.8	X	25	6
Sweet Pea	Jan.	ex	ex	ex	ex	ex	ex	...	X	25	6
Sweet Pea	Feb.	3.6	2.8	3.2	5.1	3.8	3.9	2.1	X	25	7
Aster, Crego	April	ex	ex	ex	ex	ex	ex	ex	X	30	6

a. ex symbolizes fungicide was applied as a dust in excess and the excess removed
b. X symbolizes Vancide 51 was used to just moisten the seed

weighed with an analytical balance before and after the application of each fungicide in order to determine accurately the amount adhering to the surface of the seeds.

Seeds of Phlox (Drummondii), treated by the Northrup King and Company with Arasan (8 oz./100 lbs.), Spergon (8 oz./100 lbs.), and Orthocide 75 (5.3 oz./100 lbs.), were planted in the greenhouse in December 1952. Fifty seeds per treatment pre replication with a total of eight replications were planted.

Untreated Phlox (Drummondii) seed was treated immediately before planting in January 1953 with Phygon, Arasan, Spergon, N. I. Ceresan, Orthocide 406, Red Copper Oxide, and Vancide 51. All but Vancide 51 were applied to the seed as dusts and excess dust removed. Enough Vancide 51 was applied to the seed in order to moisten the surface; the seed was dried immediately. Twenty-five seeds per treatment per replication were planted. There were eight replications.

Laboratory Procedure

The damped-off seedlings, which were brought into the laboratory were washed, surface sterilized with two parts of commercial sodium hypochlorite (Chlorox) and one part of 70 percent alcohol, rinsed in sterile distilled water, and placed in petri dishes containing water-agar acidified with a drop of lactic acid. As the fungal organism developed, hyphal tips were transferred to potato dextrose agar slants.

It was during the examination of these slants that a culture, number 1036, obtained from a damped-off Phlox seedling, was observed to be producing pycnidial imperfect and cleistothecial perfect reproductive structures. This combination of factors seemed to warrant further investigation of the organism.

From a corn meal agar subculture single spore isolations were made. Advantage of the dilution technique was made in order to isolate 17 single ascospores and 33 single conidia.

Difco potato dextrose agar was used for maintenance of all cultures and as a medium to ascertain fungal characteristics. Difco corn meal agar was used to ascertain the gross and developmental characteristics of this organism. On the later medium the mycelial growtn was limited, which permitted easier observation of pycnidial and cleistothecial development.

In observing spore germination and mycelial, pycnidial, and cleistothecial development modified Van Tieghem cells were used. These were made by sealing sterile glass rings 18 mm in diameter to sterile microscope slides with petroleum jelly and placing the slides in sterile petri dishes. Several drops of sterile distilled water were placed in the rings. Sterile 22 mm square cover glasses, coated with a thin layer of corn meal agar and inoculated with a small amount of spore suspension, were placed medium side down on the glass rings. These cultures were allowed to develop for 12 to 24 hours and then observed.

Measurements of conidia, ascospores, mycelia, and asci were made under oil immersion with a screw micrometer ocular (97 x 10X). Cleistothecia and pycnidia were measured under high dry with an ocular micrometer (10 x 43X). All dimensions given in the paper are based on fifty measurements per structure on each culture medium.

To establish the pathogenicity of culture 1036, erlenmeyer flasks (250 or 500 ml), which contained a sterilized mixture of sandy soil, were inoculated with the fungus. It was allowed to become established for two or three days and then seeds of Phlox, Aster, and Regal Lily, which had been surface sterilized by placing in a sodium hypochlorite solution in alcohol, were planted. These flasks were placed by a west window. Flasks not inoculated with the fungus were used as checks.

The diseased seedlings were treated as those coming from the field, surface sterilized, and the diseased portions planted on agar plates. The fungal organisms, which grew from the diseased material, were transferred to potato dextrose agar slants and later identified. Diseased tissue was mounted on slides in water or lacto-phenol, crushed to some extent, and observed.

Material for sectioning was fixed in F. A. A. and imbedded in paraffin. The paraffin sections were stained with Conant's Quadruple Stain.

RESULTS OF SEED TREATMENTS

The results which were obtained in the various seed treatment tests conducted over a period of 2½ years are presented in the following pages. These results are presented in tables so arranged as to give a comparison of the efficiency of the seed treatment chemicals on each ornamental variety in a particular trial or series of trials.

In Table 5 are included the results obtained in tests in which Phygon, Arasan, Spergon, and C and C 5400 were compared in the 1951 field trials. Seeds of Aster (Crego), Calendula (Double Orange King), Cosmos (Sensation), Dahlia (Unwin's Dwarf Hybrid), Four O'Clock (Marvel of Peru), Larkspur (Giant Imperial), Marigold (Harmony Dwarf), Morning Glory (Heavenly Blue), and Nasturtium (Dwarf Choice) were used. The seeds were received pre-treated by a commercial seedsman who did not treat each seed variety with every chemical.

The average temperature during this experiment was 69° F which was about 1.5° below normal for this period.[1] The precipitation was also below normal for the period, but this was supplemented by artificial means. Heavy rains did occur shortly after the plantings were made.

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1. Local Climatological Data, U.S. Dept. of Commerce, Weather Bureau, East Lansing, Michigan.

TABLE 5

EFFECT OF SEED TREATMENT ON EMERGENCE AND STANDS OF SEED TREATED WITH FUNGICIDES BY COMMERCIAL SEEDSMEN

Field trials of 1951

	Viability %	Non-treated	Phygon	Arasan	Spergon	C & C 5400
			Aster, Orego, Mixed			
% Emergence	61	25.08	28.50	26.42	24.25	...
% E. S. D-0[a]		1.33	0.88	1.58	1.72	...
% Stand		24.75	28.25	26.00	23.83	...
			Calendula, Double Orange King			
% Emergence	87	48.41	64.33**	57.17*	49.17	...
% E. S. D-0		1.02	1.36	0.58	1.03	...
% Stand		48.00	63.76**	56.83*	48.50	...
			Cosmos, Sensation			
% Emergence	93	52.38	69.50*	66.88*	53.12	...
% E. S. D-0		0.95	1.26	0.75	0.71	...
% Stand		51.87	68.62*	66.38*	52.75	...
			Dahlia, Unwin's Dwarf Hybrid, Mixed			
% Emergence	81	42.75	...	57.50	44.12	...
% E. S. D-0		0.88	...	1.30	0.86	...
% Stand		42.38	...	56.75	43.75	...
			Four O'Clock, Marvel of Peru			
% Emergence	81	70.50	...	74.00	70.00	...
% E. S. D-0		0.71	...	0	0	...
% Stand		70.00	...	74.00	70.00	...

a. Emerged seedlings damped-off.

* Significant at 5% level

** Significant at 1% level

TABLE 5 Continued

	Viability %	Non-treated	Phygon	Arasan	Spergon	C & C 5400
		Larkspur, Giant Imperial				
% Emergence	80	38.58	47.00	45.67	37.92	41.92
% E. S. D-0[a]		6.48	5.67	5.66	7.25	7.36
% Stand		36.08	44.33	43.08	35.17	38.83
		Marigold, Harmony Dwarf (French)				
% Emergence	74	61.08	60.67	58.58	59.08	55.83
% E. S. D-0		0.14	0.14	0.14	0.42	0
% Stand		61.00	60.58	58.50	58.83	55.83
		Morning Glory, Heavenly Blue				
% Emergence	61	51.50	47.50	52.75	45.00	...
% E. S. D-0		0.97	0.53	0	0	...
% Stands		51.00	47.25	52.75	45.00	...
		Nasturtium, Dwarf Choice, Mixed				
% Emergence	82	74.00	...	76.67	86.00	...
% E. S. D-0		0	...	0	0	...
% Stand		74.00	...	76.67	86.00	...

a. Emerged seedling damped-off.
* Significant at 5% level
** Significant at 1% level

The incidence of post-emergence damping-off in this planting was slight. Less than two percent of the emerged seedlings damped-off except Larkspur seedlings where not more than eight percent damped-off. Pre-emergence damping-off on the other hand was apparently more severe. Less than one-half the viable seed (according to viability percentage) emerged in the case of Aster. Emergence of Calendula, Cosmos, Dahlia, Larkspur, and Marigold was considerably below the viability level of the seed. Nasturtium seed, however, was not very susceptible to pre-emergence damping-off.

Significant improvement of the seedling emergence and stands was obtained only in the cases of the Phygon and Arasan treatments of Calendula and Cosmos seed. Although not significant, the increases due to Arasan and Spergon treatments of Dahlia and Nasturtium seed respectively were substantial. In all other cases the emergence and stand differences between the treated and non-treated seed were slight. Such differences are in the range of experimental variation and are not significant. Nevertheless, some of these small differences are indicative of trends in the value of the various chemicals as shown in subsequent experiments.

In Table 6 are included the results obtained in tests in which Phygon, Arasan, Arasan SF, Spergon, Orthocide 75, and C and C 5400 were compared on seed of Aster (American

TABLE 6

EFFECT OF SEED TREATMENT ON EMERGENCE AND STANDS OF SEED TREATED WITH FUNGICIDES BY COMMERCIAL SEEDSMEN

Field trials of 1952[a]

	Viability %	Non-treated	Phygon	Arasan	Arasan SF	Spergon	Orthocide 75	C & C 5400
Aster, American Beauty Mixed								
% Emergence	91	35.00	57.00	39.50	43.25	...	56.00	...
% E. S. D-0[b]		37.07	37.72	38.61	47.39	...	29.91	...
% Stand		22.00	35.50	24.25	22.75	...	39.25	...
Bachelor Button								
% Emergence	83	16.17	52.50**	42.33**	36.50**	27.00	52.33**	...
% E. S. D-0		3.09	2.22	1.97	1.83	2.47	4.46	...
% Stand		15.67	51.33**	41.50**	35.83**	26.33*	50.00**	...
Bachelor Button (September planting)								
% Emergence	83	9.50	23.00**	20.50*	21.75**	11.50	24.50**	...
% E. S. D-0		7.89	2.17	2.44	5.75	8.70	8.16	...
% Stand		8.75	22.50**	20.00**	20.50**	10.50	22.50**	...
Calendula, Double Orange King								
% Emergence	89	20.75	45.50**	27.50	26.25	23.50	27.50	32.25*
% E. S. D-0		45.80	46.70	51.82	62.86	53.19	54.55	51.16
% Stand		11.25	24.50	13.25	9.75	11.00	12.50	15.75
Cosmos, Sensation								
% Emergence	85	46.75	58.25*	53.75	62.25*	46.75	58.50*	58.75*
% E. S. D-0		1.60	3.00	8.33	4.82	4.28	2.14	3.83
% Stand		46.00	56.50*	49.00	59.25*	44.75	57.25*	56.50*

a. Trials run in summer unless otherwise designated
b. Emerged seedlings damped-off

* Significant at 5% level
** Significant at 1% level

TABLE 6 Continued

	Viability %	Non-treated	Phygon	Arasan	Arasan SF	Spergon	Orthocide 75	C & C 5400
Dahlia, Unwin's Dwarf Hybrid, Mixed								
% Emergence	90	24.50	34.25	29.25	35.00	29.00	27.00	27.00
% E. S. D-0[b]		18.37	33.58	17.09	14.29	23.38	13.89	30.40
% Stand		20.00	23.50	24.25	30.00	22.25	23.25	19.75
Four O'Clock, Marvel of Peru								
% Emergence	90	33.00	50.00*	55.33**	...	50.00*	60.50**	44.50
% E. S. D-0		0	7.00	0	...	4.00	4.13	2.25
% Stand		33.00	46.50	55.33*	...	48.00	58.00*	43.50
Larkspur, Double Mixed								
% Emergence	84	27.67	30.50	37.83	...	33.83	31.00	31.50
% E. S. D-0		30.12	21.86	18.50	...	13.72	30.65	24.34
% Stand		19.33	23.83	30.82	...	27.50	21.50	23.83
Marigold, African								
% Emergence	87	39.75	43.00	42.50	...	41.25	41.75	43.25
% E. S. D-0		1.26	0.59	0.59	...	1.21	2.39	0.53
% Stand		39.25	42.75	42.25	...	40.75	40.75	43.00
Morning Glory, Heavenly Blue								
% Emergence	56	28.67	44.00*	41.33*	...	37.00*	37.33*	...
% E. S. D-0		11.65	8.30	10.48	...	8.11	12.50	...
% Stand		25.33	40.33*	37.00*	...	34.00	32.67	...

b. Emerged seedlings damped-off

* Significant at 5% level
** Significant at 1% level

TABLE 6 Continued

	Viability %	Non-treated	Phygon	Arasan	Arasan SF	Spergon	Orthocide 75	C & C 5400
				Nasturtium, Glor. Gleam Hybrid, Mixed				
% Emergence	60	42.00	64.00*	50.00	...	45.00	63.00*	...
% E. S. D-0[b]		4.79	7.81	0	...	4.44	7.94	...
% Stand		40.00	59.00	50.00	...	43.00	58.00	...
				Zinnia, Giant of California, Mixed				
% Emergence	92	48.25	60.00	60.50	65.25	...	63.25	...
% E. S. D-0		21.24	24.17	13.22	31.03	...	15.11	...
% Stand		38.00	45.50	52.25	45.00	...	53.25	...
				Zinnia, Giant of California, Mixed (September planting)				
% Emergence	92	34.75	54.50**	57.50**	58.75**	...	60.50**	...
% E. S. D-0		28.06	27.06	20.00	13.62	...	26.03	...
% Stand		25.00	39.75**	46.00**	50.75**	...	44.75**	...

	Viability %	Non-treated	Phygon	N. I. Ceresan	Arasan SF	Vancide 51	Orthocide 406	C & C 5400
				Sweet Pea				
% Emergence	80	38.50	58.25*	50.50	38.00	31.75	39.50	35.00
% E. S. D-0		16.23	33.48	37.62	40.13	36.22	34.18	45.00
% Stand		32.25	38.75	31.50	27.75	20.25	26.00	19.25

b. Emerged seedlings damped-off

* Significant at 5% level
** Significant at 1% level

Beauty), Bachelor Button, Calendula (Double Orange King), Cosmos (Sensation), Dahlia (Unwin's Dwarf Hybrid), Four O'Clock (Marvel of Peru), Larkspur (Double Mixed), Marigold (African), Morning Glory (Heavenly Blue), Nasturtium (Glor. Gleam Hybrid), Zinnia (Giant of California), and Sweet Pea. With the exception of Sweet Pea the seeds were pre-treated. As in the 1951 trials not every variety was treated with each chemical. All varieties were used in the May-June trials. In the fall planting only Bachelor Button and Zinnia were used. The seeds used in the second planting were from the same lots as were used in the summer plantings. In the summer trials Calendula, Cosmos, Dahlia, Four O'Clock, Marigold, Morning Glory, Nasturtium, and Zinnia were planted during the third week of May and Aster, Larkspur, and Sweet Pea during the first week of June.

The temperature was a little above normal during the summer experiment with an average during the entire period of 68° F. The last part of May was cool, however, with an average of 58° F. The average temperature of June and the first half of July was 72° F; this was about three degrees above normal. The cool May weather occurred in connection with heavy precipitation in that period, while it was dry during June and July. Of course, as pointed out earlier, a high soil moisture level of the plot was maintained by a sprinkler system. The average temperature during the fall

experiment was 64° F. The soil moisture was high during the first week because of considerable precipitation right after the planting of the seed.

Damping-off was much more severe than in the previous year. In one case, post-emergence damping-off caused a loss of 63 percent of the emerged seedlings. In many instances these post-emergence losses were more than 10 percent. Emergence in relation to seed viability was lower in 1952, indicating that the incidence of pre-emergence damping-off was also greater. Although susceptibility to damping-off was not studied, some differences in post-emergence susceptibility were apparent.

Seedling emergence of Phygon-treated seeds was significantly improved over the non-treated seeds of Bachelor Button (both plantings), Calendula, Cosmos, Four O'Clock, Morning Glory, Nasturtium, Sweet Pea, and the fall planting of Zinnia. Although not significant, noteworthy emergence improvement due to the Phygon treatment of Aster, Dahlia, and Zinnia (summer planting) seed was produced. Seedling emergence of Larkspur and Marigold was only slightly improved by the Phygon treatment.

Seedling emergence of Bachelor Button (both plantings), Four O'Clock, Morning Glory, and Zinnia (fall planting) seed treated with Arasan was significantly better than the non-treated. Substantial improvement was also noted in the case of Larkspur and Zinnia (summer planting). In every

other case only slight improvement of the average emergence occurred.

The performance of Arasan SF was generally similar to Arasan, wherever they were both used. The notable exception was to be found with Cosmos, where the emergence of Arasan SF-treated seed was significantly better than the Arasan. Although significant differences did not exist, the Arasan SF treatment was the best chemical used on Dahlia seed.

Spergon as a seed treatment chemical generally was of little value except for Four O'Clock and Morning Glory. Substantial improvement of the emergence and final stand of Bachelor Button seedlings were produced in the summer planting, but not in the fall trial. Since Spergon was found to be of little value on Aster and Sweet Pea in earlier trials it was not used on these species in this trial.

Orthocide 75-treated seed emerged significantly better in the case of Bachelor Button (both plantings), Cosmos, Four O'Clock, Morning Glory, Nasturtium, and Zinnia (fall planting). It was also an outstanding chemical on Aster and Zinnia (summer planting). In other instances, that is, Calendula, Larkspur, and Marigold, emergence of Orthocide 75-treated seed was little better than the non-treated.

C and C 5400 as a seed treatment chemical proved to be of significant value only on Calendula and Cosmos seed. It did substantially improve emergence of Four O'Clock seed,

but this was still inferior to other treatments.

Aside from Phygon only N. I. Ceresan proved to be of any value for treating Sweet Pea seed to increase emergence in this summer trial.

The frequency of the different fungi causing post-emergence damping-off was determined for the damped-off Zinnia seedlings of the fall planting. When the organisms were allowed to grow out of diseased tissue on agar plates the following percentages of the different fungi developed: Rhizoctonia solani 65, Fusarium spp. 11, and various other fungi 24. Some of the various fungi were: Penicillium, Alternaria, Rhizopus, Hormodendron, Helminthosporium, Trichoderma, Phoma.

The results of the tests in which Phygon, Arasan, Spergon, and C and C 5400 were tested on seeds of Aster (Crego), Calendula (Double Orange King), Larkspur (Giant Imperial), and Marigold (Harmony Dwarf) in the greenhouse tests of 1951 are given in Table 7. These seeds were from the same lots of seeds of these species used in the summer 1951 trials. The percentage of seedling emergence for all four varieties was in the general range of that obtained in the summer trials.

The Phygon and Arasan treatments were significantly effective in improving both emergence and stands of seeds of Aster and Calendula. Spergon although significantly better than the non-treatment was still significantly poorer than

TABLE 7

EFFECT OF SEED TREATMENT ON EMERGENCE AND STANDS OF SEED TREATED WITH FUNGICIDE BY COMMERCIAL SEEDSMEN

Greenhouse trials of 1951

	Viability %	Non-treated	Phygon	Arasan	Spergon	C & C 5400
			Aster, Crego, Mixed			
% Emergence	61	9.33	25.00**	26.33**	15.67*	...
% E. S. D-0[a]		3.57	5.33	2.53	12.77	...
% Stand		9.00	23.67**	25.67**	13.67	...
			Calendula, Double Orange King			
% Emergence	87	32.33	77.33**	73.00**	43.33*	...
% E. S. D-0		6.18	1.72	1.37	0.77	...
% Stand		30.33	76.00**	72.00**	43.00*	...
			Larkspur, Giant Imperial			
% Emergence	80	47.00	50.67	46.50	42.16	41.33
% E. S. D-0		1.06	0.97	1.08	3.16	1.61
% Stand		46.50	50.17	46.00	40.83	40.67
			Marigold, Harmony Dwarf (French)			
% Emergence	74	56.67	60.33	61.33	56.33	54.33
% E. S. D-0		1.76	2.76	0	2.37	1.23
% Stand		55.67	58.67	61.33	55.00	53.67

a. Emerged seedlings damped-off
* Significant at 5% level
** Significant at 1% level

Arasan and Phygon with Aster and Calendula. Larkspur and Marigold did not respond to any degree to the treatments in this trial, and some treatments were even slightly inferior to the non-treated check.

In Table 8 are presented the data from a series of experiments conducted in the greenhouse during the early months of 1952. In this series of trials untreated seed was obtained and treated by the author with Phygon, Arasan, Spergon, C and C 5400, N. I. Ceresan, Red Copper Oxide, and Vancide 51. Orthocide 406 was also used in three cases. The ornamentals used were: Calendula (Double Orange King), Stock, in two experiments, Sweet Pea, in two experiments, and Aster (Crego).

The seedling emergence of Calendula was substantially improved by all seed treatment chemicals. Spergon was the least effective. Some of the treatments nearly doubled the seedling emergence and final stands. Vancide 51, Phygon, C and C 5400, Arasan, Red Copper Oxide, and N. I. Ceresan, in that order, were all highly significant treatments. About a 24-hour delay in germination was caused by the Ceresan. This delay, however, was only temporary, so that by the end of the experiment the seedlings were as large and vigorous as any others.

Seed treatment of Stock seed benefited the emergence and stands as shown by the two successive trials. The same seed was used in both trials. The N. I. Ceresan treatment

TABLE 8

EFFECT OF SEED TREATMENT ON EMERGENCE AND STANDS OF SEED TREATED WITH FUNGICIDES BY THE AUTHOR

Greenhouse trials in the Winter of 1952

	Viability %	Non-treated	Phygon	Arasan	Spergon	C & C 5400	N. I. Ceresan	Red Copper Oxide	Vancide 51	Orthocide 406
Calendula, Double Orange King										
% Emergence	95	46.67	90.67**	85.33**	56.00*	88.00**	72.67**	82.00**	90.00**	...
% E. S. D-0[a]		7.14	3.79	6.25	11.90	2.94	3.67	5.69	1.48	...
% Stand		43.33	88.00**	80.00**	49.33	84.67**	70.00**	77.33**	88.67**	...
Stock										
% Emergence	93	59.00	77.00*	73.50*	51.00	56.00	80.50**	74.00**	76.50*	...
% E. S. D-0		20.34	7.14	10.94	27.45	10.71	3.11	9.46	3.27	...
% Stand		47.00	71.50**	65.50**	37.00	50.00	78.00**	67.00**	74.00**	...
Stock										
% Emergence	93	72.00	80.67	86.67*	80.67	88.67*	85.33*	84.00*	82.67*	83.33*
% E. S. D-0		1.85	3.31	8.46	4.13	7.51	2.34	1.59	1.61	2.60
% Stand		70.67	78.00	79.33	77.33	82.00	83.33	82.67	81.33	75.33

a. Emerged seedlings damped-off
* Significant at 5% level
** Significant at 1% level

TABLE 8 Continued

	Via-bility %	Non-treated	Phygon	Arasan	Spergon	C & C 5400	N. I. Ceresan	Red Copper Oxide	Vancide 51	Ortho-cide 406
					Sweet Pea					
% Emergence	92	23.33	85.33**	35.33	28.67	69.33**	85.33**	54.00**	70.00**	...
% E. S. D-0[a]		17.14	17.97	28.30	30.23	33.65	7.81	4.94	31.43	...
% Stand		19.33	70.00**	25.33	20.00	46.00*	78.67**	51.53**	45.00*	...
					Sweet Pea					
% Emergence	92	20.57	67.43**	39.43**	28.86	58.86**	66.86**	36.00**	56.00**	58.29**
% E. S. D-0		38.89	40.68	44.93	58.00	34.95	54.70	22.22	39.79	21.57
% Stand		12.57	40.00**	21.71	12.00	38.29**	30.29**	28.00*	33.71**	45.71**
					Aster, Crego, Mixed					
% Emergence	96	55.55	74.44**	78.89**	63.33	76.11**	77.22**	67.22	77.78**	77.78**
% E. S. D-0		18.00	16.41	10.56	14.04	15.33	35.25	13.25	25.00	10.71
% Stand		45.55	62.22	70.55	54.44	64.44	50.00	58.33	56.33	69.44

a. Emerged seedlings damped-off
* Significant at 5% level
** Significant at 1% level

was about the best in each instance. In the first trial N. I. Ceresan, Vancide 51, Phygon, Red Copper Oxide, and Arasan treated seed in that order, produced emergence and stands significantly better than the non-treated control and the Spergon and C and C 5400 treated seed. The Spergon treatment was considerably poorer than no treatment. In the second trial where approximately the same amount of each fungicide was used as in the earlier trial the results were somewhat more variable. All chemicals used improved emergence and final stands; however, emergence of Phygon- and Spergon-treated seed was not significantly better than that of the non-treated. The treatments giving significant improvement in seedling emergence were: C and C 5400, N. I. Ceresan, Red Copper Oxide, Orthocide 406, and Vancide 51 in that order.

The relative occurrence of the different fungi causing post-emergence damping-off was determined for the second trial of Stock. When the organisms were allowed to grow out of the diseased tissue on agar plates the following percentages of different fungi developed: Rhizoctonia solani 34, Pythium spp. 14, Fusarium spp. 4, various other molds 11, and none 37.

Sweet Pea seed, like Stock, was used in two successive trials, in which approximately the same amount of each chemical was used in each trial. Not only were the results from chemically treated seed significantly better than the non-treated, but certain chemicals were significantly better

than others. Although some variation in the results was evident between the experiments, in the main the results were complementary. Since post-emergence damping-off was not controlled by the chemicals used, conclusions are based on the emergence data and protection against pre-emergence damping-off. Based on the results obtained in these two trials the chemical treatments fall into four groups: 1. Phygon and N. I. Ceresan; 2. Vancide, C and C 5400, and Orthocide 406; 3. Red Copper Oxide and Arasan; and 4. Spergon, from the best to the poorest in that order. The Spergon treatment was little better than the non-treated. The Arasan treatment gave significantly better emergence only in the second trial, while Red Copper Oxide proved to be better in both trials. The C and C 5400, Vancide 51, and Orthocide 406 treatments gave about equal pre-emergence protection. Phygon and N. I. Ceresan were superior to all other treatments and gave protection significantly better than some other treatments. For example, these treatments were significantly better than all others in the first trial. This superiority was still found in the second trial, but the differences were not quite so great.

No chemical injury of the Sweet Pea was apparent, except that some Red Copper Oxide treated seeds were slow to germinate. When these seeds were dug up many could be found that had not swollen and were still well-coated with the chemical. Once the seed became swollen by the uptake of

water, it would germinate readily. This would indicate that the Red Copper Oxide coating on these seeds coupled with the hard seet coat prevented the entrance of moisture into the seed and thus delayed or even in some cases prevented germination.

The relative occurrence of the different fungi that grew out of a few of the damped-off Sweet Pea seedlings was determined in the first experiment. The following percentages of the different fungi that developed are: Pythium spp. 70, Rhizoctonia solani 7, Fusarium spp. 8, and other various fungi 14. In the second trial with Sweet Pea all the diseased seedlings were checked. The following fungi were found: Rhizoctonia solani 60, Pythium spp. 14, Fusarium spp. 3, other various fungi 15, and none 8. Thus, on the same soil, the relative prevalence of the damping-off fungi differed from that in the previous experiment.

In the experiment in which Aster was used all chemical treatments proved to give better results than did the non-treatment. Emergence of Spergon and Red Copper Oxide treated seed were not significantly better than the non-treated, however, Arasan, Orthocide 406, Vancide 51, N. I. Ceresan, C and C 5400, and Phygon, all about equally effective, were significantly better. Although final stand differences were not significant, the final seedling stands were superior where the chemical treatments of the seed were used.

In Table 9 are presented the results of two successive greenhouse experiments using Phlox (Drummondii) seed. Two earlier experiments using Phlox in the field had been washed out so badly that it was impossible to read the results. In the first experiment in December of 1952 the seed was pre-treated with Arasan, Spergon, and Orthocide 75. For the second experiment in January 1953 untreated seed was treated by the author with Phygon, Arasan, Spergon, N. I. Ceresan, Red Copper Oxide, Orthocide 406, and Vancide 51.

Arasan and Orthocide 75 increased the number of emerging seedlings significantly over the non-treatment and the Spergon treatment in the first trial. In the second experiment differences were not statistically significant although some of them appeared substantial. Orthocide 406, N. I. Ceresan, Arasan, and Red Copper Oxide were the better seed treatment chemicals. Phygon, Spergon, and Vancide 51 were of no real value. It is noteworthy that the performances of Arasan and Orthocide (Orthocide 75 and Orthocide 406 have the same active ingredient but contain 75 and 50 percent respectively) treatments were about equally effective in the two experiments.

The fungi causing seedling damping-off were determined for some of the diseased Phlox seedlings in the December trial. It was found that 86 percent of these seedlings yielded Rhizoctonia solani and 14 percent various other fungi. One of the various other fungi when studied further

TABLE 9

EFFECT OF SEED TREATMENT ON EMERGENCE AND STANDS OF SEED TREATED WITH FUNGICIDES

Greenhouse trials in Winter 1952-53

	Viability %	Non-treated	Phygon	Arasan	Spergon	N. I. Ceresan	Red Copper Oxide	Orthocide	Vancide 51
				Phlox, Drummondii, Mixed					
% Emergence	85	58.50	...	69.75**	62.75	...	...	72.75**[b]	...
% E. S. D-O[a]		8.12	...	5.73	6.77	...	...	12.03	...
% Stand		53.75	...	65.75*	58.50	...	...	64.00*	...
				Phlox, Drummondii, Mixed					
% Emergence	85	61.50	63.00	69.50	63.00	73.00	68.00	73.00[c]	61.00
% E. S. D-O		8.94	19.84	5.75	6.35	4.79	5.15	6.16	7.38
% Stand		56.00	50.50	65.50	59.50	69.50	64.50	68.50	56.50

a. Emerged seedlings damped-off
b. Orthocide 75 (75% active)
c. Orthocide 406 (50% active)
* Significant at 5% level
** Significant at 1% level

was found to be a new damping-off causing organism. This study is reported in another section of this paper.

SEED TREATMENT DISCUSSION

Chemical seed treatment as a means of checking pre-emergence damping-off of ornamentals has proved to be beneficial for many of the species tested in these trials. The protection varied not only from chemial to chemical but also from species to species. The relative values of the fungicides tested on the different ornamentals included in these experiments are shown in Table 10. The fungicides are rated as superior, promising, doubtful, and of no value, according to the emergence data only.

In those species where two varieties were used as in the trials with Aster (Appendix Figs. 1-4), Larkspur (Appendix Figs. 17-19), and Marigold (Appendix Figs. 20-22) the relative value of each fungicide did not vary from variety to variety. However, under the conditions of these experiments these differences were generally similar to the differences between the various lots of seed of the same variety as in trials with Calendula (Appendix Figs. 5-8) and Sweet Pea (Appendix Figs. 29-31). Each variety has been treated separately in Table 10.

From this table it can be seen that some chemicals were generally more effective as ornamental seed protectants than others. The most consistently valuable fungicide was Phygon, while Spergon was the poorest. Even when the dosage

TABLE 10

COMPOSITE RATING OF THE SEED-TREATMENT FUNGICIDES BASED ON EMERGENCE FOR EACH ORNAMENTAL VARIETY

Ornamental variety	Superior	Promising	Doubtful	No value
Aster, Crego		Phygon Arasan N.I.Ceresan Vancide 51 C & C 5400 Ortho. 406	Red Cu0[a] Spergon	
Aster, American Beauty		Phygon Ortho. 75	Spergon Arasan SF Arasan	
Bachelor Button	Phygon Ortho. 406	Arasan Arasan SF	Spergon	
Calendula, Double Orange King	Phygon	Arasan Vancide 51 C & C 5400 Red Cu0[a] N.I.Ceresan	Spergon Ortho. 75 Arasan SF	
Cosmos, Sensation		Phygon Arasan SF C & C 5400 Ortho. 75 Arasan		Spergon
Dahlia, Unwin's Dwarf Hybrid		Arasan Arasan SF Phygon	Spergon C & C 5400 Ortho. 75	
Four O'Clock, Marvel of Peru		Arasan Ortho. 75 Phygon Spergon	C & C 5400	
Larkspur, Giant Imperial		Phygon	Arasan	Spergon C & C 5400

a. Red Copper Oxide

TABLE 10 Continued

Ornamental variety	Superior	Promising	Doubtful	No value
Larkspur, Double Mixed		Arasan	Phygon Spergon C & C 5400 Ortho. 75	
Marigold, Harmony Dwarf			Phygon Arasan	Spergon C & C 5400
Marigold, African			C & C 5400 Phygon Arasan Ortho. 75 Spergon	
Morning Glory, Heavenly Blue		Phygon Arasan Spergon Ortho. 75		
Nasturtium, Dwarf		Spergon	Arasan	
Nasturtium, Glor. Gleam Hybrid		Phygon Ortho. 75	Spergon Arasan	
Phlox, Drummondii		Ortho. 75 Ortho. 406 Arasan N.I.Ceresan Red CuO[a]	Phygon Spergon	Vancide 51
Stock		N.I.Ceresan Arasan Vancide 51 Red CuO[a] Phygon C & C 5400 Ortho. 406	Spergon	
Sweet Pea	Phygon N.I.Ceresan	Vancide Ortho. 406 C & C 5400	Red CuO[a] Arasan Arasan SF	Spergon
Zinnia, Giant of California	Ortho. 75 Arasan SF	Arasan Phygon		

a. Red Copper Oxide

of Spergon was increased it was still a poor treatment. On only two ornamentals, namely Morning Glory and Nasturtium, did the Spergon treatment respond at all favorably. Arasan was generally promising, but did not always measure up to Phygon. In some instances, however, it was somewhat better. Arasan SF was of no more value than Arasan. N. I. Ceresan was a very promising seed protectant, however, its potentialities were not fully investigated in these experiments. It is reported to be injurious on some plants. The Orthocides, even though of different concentrations, were generally effective in pre-emergence control. Red Copper Oxide, used to only a limited degree, was of some value, but generally was outranked by some of the organics. Vancide 51 was of value where used.

Marigold was the only ornamental on which the seed protectants failed to give some measure of protection against pre-emergence damping-off. Only limited protection was apparent on Larkspur and Dahlia. Perhaps higher dosages of the fungicides would have given more protection. In contrast the most noticeable protection was provided Sweet Pea against pre-emergence damping-off when seed protectants were used.

The experimental data shown in graphic form (Appendix Figs. 1-35) do not in any case indicate that any one chemical contributes much toward post-emergence damping-off control. This is best illustrated by the two successive Sweet Pea trials (Appendix Figs. 29-30). The emergence data in the two

experiments are very well correlated even though damping-off was more severe in the second trial. The stand data, on the other hand, were conflicting.

Tisdale et al. (44) reporting on dust treatments for vegetable seed, also concluded that, "Seed treatments provide little protection against post-emergence damping-off, even though they are very satisfactory in preventing seed decay."

The post-emergence damping-off susceptibility of the ornamentals studied in these experiments may be rated by species as: very susceptible - Aster and Sweet Pea; moderately susceptible - Calendula, Dahlia, Larkspur, Phlox, Stock, and Zinnia; slightly susceptible - Bachelor Button, Cosmos, Four O'Clock, Morning Glory, and Nasturtium; and semi-resistant - Marigold.

Damping-off generally became more severe with each successive use of the same soil in both greenhouse and the field (Appendix Figs. 1-35). Environment in the field could have been partly responsible for this, but it would seem more logical to consider this a function of inoculum potential. Inoculum potential was enhanced in both the field and the greenhouse by soil inoculations, but in the greenhouse where the soil was used a second and even a third time the damping-off was invariably higher on successive trials. This was well illustrated by the Sweet Pea trials (Appendix Figs. 29-30). Since the inoculum potential tends to increase in successive plantings on the same soil, the use of fresh soil

in the greenhouse and rotation in the field would tend to lessen damping-off. This would be control through escape.

A partial check of the parasites responsible for post-emergence damping-off was made and it was found that the organisms responsible changed from one trial to the next. Although exact data were not taken in the 1951 field trials the organisms primarily responsible for post-emergence damping-off were pythiaceous. In the 1952 fall experiment it was primarily Rhizoctonia which was causing the seedling damping-off. Temperature differences for the different trials hardly would account for this since the Pythium spp. are favored by cooler temperatures and it was the Rhizoctonia that caused the damage in the fall trial. This change was also evident in the greenhouse with the Sweet Pea trials where the temperature and moisture conditions were more uniform.

Several lines of further research are suggested by these experiments. Further investigation of the dosages required for each of the better chemicals for each species is suggested. Expand the investigation to include more ornamental species, always keeping in mind that the commercial seedsmen will use such information for pre-treating practices. Study post-emergence control procedures. Through controlled inoculation and reisolation experiments of the various damping-off fungi establish the host range of these pathogens on ornamentals. Investigate damping-off and control methods in relation to environmental factors.

AN ASPERGILLACEOUS FUNGUS CAUSING DAMPING-OFF

In the laboratory, while studying a group of cultures isolated from damped-off Phlox seedlings, an unidentifiable fungus, number 1036, was found.

Pathogenicity

Proof of the ability of this organism to cause post-emergence damping-off was established on Aster, Phlox, and Regal Lily. Seedlings of these varieties, when grown in sterile erlenmeyer chambers inoculated with the fungus, often became diseased. Symptoms of the disease were typical of damping-off with a water-soaked appearance of the stem at or just below the soil line. If the seedling was allowed to remain in the chamber then this water-soaked appearance gradually progressed upward in the stem. Infection in the cotyledonary leaves showed the water-soaking and chlorosis. In every case where these diseased seedlings were plated out on water agar, an organism, similar in every aspect to the parent culture, was reisolated. Microscopic examination of the diseased host tissue revealed the hyphal strands permeating through the tissues and even some evidence of cell wall penetration (Pl. IV, Fig. 22).

Description of the Organism

Gross laboratory and microscopic observations of the culture, number 1036, revealed features which placed it in no known genus. By making single spore isolations of 17 ascospores and 33 conidia pure strains, identical to the original isolate, were obtained for further study.

Because of the astomous and firm-walled fructification, with globose asci scattered irregularly throughout the cavity, the organism has been tentatively placed in the Aspergillaceae. It differs from all other members of this family by the possession of an imperfect stage, a pycnidium. It is from this characteristic of the organism that the generic name was chosen: Pycnidiophora, derived from the Greek: Pyknon - idion, and phoreus; and literally meaning "bearer of pycnidia".

Pycnidiophora gen. nov.

Cleistothecia immersa vel semiimmersa, per substratum dispersa, globosa, immatura translucentia, matura fusca et opaca, exostiolata, pariete membranaceo, fragili et exappendiculato; hyphae immersae vel aeriae ad substratum appressae; ascis globosis, tenuibus, delicatis, evanescentes, brevipedicellatis, aparaphysatis, continentibus trigintaduo sporas; sporis conglobatis, simplicibus, oblongo-reniformis et pallide brunneis; status imperfectus pycnidialis.

Cleistothecia submerged to partially superficial and scattered throughout substrate, globose, translucent when young, dark and opaque when mature, astomous; cleistothecial wall membranous, brittle and without appendages. Hyphae either submerged or, when aerial, usually closely appressed to substrate surface, the exact nature dependent upon substrate. Asci globose, thin-walled, delicate, evanescent, short-stalked when immature, without paraphyses and with 32 spores. Spores conglobate, unicellular, oblong-reniform and light brown in color. Reproductive bodies of imperfect stage pycnidial.

Type species, Pycnidiophora dispersa

Pycnidiophora differs from the genus Fragosphaeria Shear by having brown ascospores and a brown, instead of a purple, cleistothecial wall. Although it resembles the genera Magnusia Sacc., Arachnomyces Massee and Salm., and Cephalotheca Fuck., the cleistothecia of this organism do not have appendages. The brown cleistothecium distinguishes this entity from the genus Laaseomyces Ruhl. Conglobate and oblong-reniform spores separate it from the genus Thielavia Zopf. which produces elliptic spores.

Because of the scattered or dispersed nature of the cleistothecia the name dispersa, from the Latin: dispergere to scatter, was chosen.

Pycnidiophora dispersa sp. nov.

Mycelia homothallica, multiramosa; hyphis 1-6 u in diametro; cleistothecia 60-700 u in diametro; ascis globosis, 10-14.5 u in diametro; sporis 2.0-2.9 x 2.8-5.8 u, immaturis hyalinis et maturis pallide brunneis; pycnidia superficialia, super substratum dispersa, globosa ad irregulariter-elongata, glabra, in parte inferiore incolorata, nisi cellulae circa leviter papillatum ostiolum pallide brunneae; 26-78 x 26-164 u in diametro; conidiophoris simplicibus, brevissimis; conidia hyalina, simplicia, oblonga, 1.5-3.4 x 2.6-4.75 u.

Mycelia homothallic, profusely branched, the hyphae 1-6 u in diameter; cleistothecia 60-700 u in diameter; asci globose 10-14.5 u in diameter. Ascospores 2-2.9 x 2.8-5.8 u, hyaline when young, later becoming light brown. Pycnidia scattered on surface of substrate, varying from globose to irregular-elongate in shape, glabrous; the lower portion colorless and the walls of the cells around the slightly papillate ostiole light brown, 26-78 x 26-164 u in size. Conidiophores simple, extremely short. Conidia hyaline, unicellular, oblong, 1.5-3.4 x 2.6-4.75 u.

Type specimen: Floyd M. Clum 27 from cultures prepared during study of the organism, deposited in the Beal-Darlington Herbarium of Michigan State College (accession number 133,118); isotype specimens also deposited in the University of Michigan and University of Wisconsin herbaria, and cultures to be sent to the type culture collections in American Type

Culture Collection, Washington, D.C. and Centraalbureau Voor Schimmelcultures, Baarn, Netherlands.

Spore Germination and Vegetative Development

The first visible signs of ascospore germination (Pl. IV, Fig. 1) required 10 to 12 hours to appear. The spore wall apparently broke open on the flat or concave side and a bulbous swelling developed (Pl. IV, Figs. 2 and 3). This swelling enlarged to approximately three times the size of the original spore. Then a protrusion arose on one side of the swelling (Pl. IV, Fig. 4). This protrusion elongated into a germ tube. Generally another tube was produced from the opposite side of the bulbous structure at a later time, although this second tube could develop at any time (Pl. IV, Figs. 4 and 5a). The germ tube was observed to branch at any time but usually not until it had reached considerable length (Pl. IV, Figs. 5, 6, and 7). Once the hyphae began to develop, branching occurred quite frequently and gave rise to a loose spreading colony with few aerial hyphae.

Large hyphae could be traced from the center of the colony to the edge. These larger hyphal strands were dominant both on the surface and submerged and they shall be designated throughout this discussion as primary hyphae. Branches from these hyphae generally arose at right angles to the parent strand and usually were smaller in diameter;

the subsequent branches were progressively smaller, and the ultimate branch was often less than a micron in diameter. These smaller branches shall be designated as secondary hyphae.

Frequent anastomosing was observed. This occurred between two primary hyphae lying side by side, or by the fusion of the tips of secondary hyphae, or by the fusion of the end of a secondary hypha and a primary hypha.

The diameter of the hyphae measured from 0.8-6.0 u on potato dextrose agar and from 1.0-5.5 u on corn meal agar (Table 11). Primary hyphal averages were 4.2 u on potato dextrose agar and 4.0 u on corn meal agar. On potato dextrose agar the secondary hyphae averaged 2.1 u in diameter and on corn meal agar they averaged 1.9 u. In the Van Tieghem cell a hyphal strand was found to grow at the rate of 2.8 u per minute or 1.7 mm per hour over a four-hour period.

The homothallic condition of Pycnidiophora dispersa could be seen in the single spore isolations. In all fifty isolations the ascocarps were produced as abundantly as in the original culture.

When grown on corn meal agar the colony was thin, spreading, whitish, or translucent, submerged and superficial with little aerial hyphae. After the pycnidia were produced the surface of the colony had a slimy appearance because of conidial production. As the colony aged the blackish cleistothecia developed, scattered or dispersed, throughout the medium and gave the colony a speckled appearance.

Upon potato dextrose agar the total characteristics of the colony were very similar to those found in cultures on corn meal agar except that the growth was more dense, the colony color was a dull gray to buff, and more aerial hyphae were produced. The slimy appearance was lacking since the aerial hyphae covered the pycnidia. Cleistothecia were similar in appearance, but larger and more abundant.

Germination of the oblong conidium (Pl. IV, Fig. 8) started almost immediately after placement on the agar. This was in the form of a swelling of the spore (Pl. IV, Fig. 9). In three to four hours the spore was nearly globose at which time a small protrusion developed on the surface at one end much as the yeast cell produces a bud (Pl. IV, Fig. 10).

The bud elongated and at the same time a second bud usually began to develop on the opposite side although in many cases the development of the second bud was delayed until the first germ tube was well developed (Pl. IV, Figs. 10-13). In one case a third germ tube was seen to develop from the spore (Pl. IV, Fig. 12). Branching occurred at any time but seemed to delay until the tube reached considerable length. Hyphal development from a conidium was the same as from the ascospore (Pl. IV, Figs. 14 and 15).

Pycnidial Development

The small ostiolate pycnidia were glabrous, varied from globose to irregular-elongate in shape, and were 26-78 x 23-164 u on potato dextrose agar and 26-53 x 26-95 u on corn meal agar (Table 12).

During the development of the imperfect or pycnidial stage, the primordia could be observed within the first 24 hours after inoculation of the agar and mature pycnidia within the first 36 hours. All stages of development from mature pycnidia on old mycelium to beginning stages on the younger mycelium, could be seen even in a small sector of a colony. They were produced in moderate abundance primarily along the agar surface, but in the very young colonies pycnidia would be produced within the agar. The pycnidial primordia were initiated by the cutting-out of several short cells along a hyphal strand. This hyphal strand was either a primary hypha (Pl. V, Figs. 23 and 24) or a secondary hypha (Pl. V, Figs. 26, 27, and 31), but more commonly the latter.

One or two of the center cells of this initial then became enlarged (Pl. V, Figs. 23 and 24), underwent two or three transverse divisions, and gave rise to a three- to six-celled structure (pl. V, Figs. 25 and 26). This structure, by continued swelling, and by division in all planes, formed a roundish mass with very few or no hyphal branches (Pl. V, Figs. 27-29, 31-33). As this mass continued

to enlarge the upper portion, because of unequal growth, protruded slightly as a short rostrum. This was the primordium which became a flask-shaped pycnidium by the formation of a small ostiole around the apex. The walls of the cells around the ostiole and a few adjacent cells became brown, thus giving the nearly colorless pycnidium a brownish appearance. As soon as the ostiole appeared conidia were exuded out over the surface of the pycnidium (Pl. V, Figs. 30, 33-34) or accumulated as a sizable droplet at the apex of the pycnidium. In any case the mature pycnidium originated from a single hypha.

Certain variations in pycnidial development occurred. Pycnidia with two or even three ostioles were commonly observed. This, perhaps, was due to either the activation of a large number of cells in continuous series, or to the development of two or three initials very close together on the same hyphal strand. In only one instance was pycnidial development observed to be compound meristogenous. This occurred when a considerable amount of aerial hyphae grew out of a small portion of infected plant tissue that had been placed on water agar. On these hyphae developed a number of pycnidia which were darker in color than those described above and, on microscopic examination, appeared to have been derived from two to four hyphal strands that had been growing side by side.

The hyaline conidia were produced on simple, extremely short conidiophores lining the inner portion of the pycnidium (Pl. IV, Fig. 21). These conidia filled the pycnidial cavity and at maturity were forced out in droplets that had a milky appearance and gave the surface of the agar around the pycnidia a slimy consistency. The conidia were oblong, very small, 1.5-3.4 x 3.1-4.8 u on potato dextrose agar and 1.8-3.0 x 2.6-4.8 u on corn meal agar, unicellular, and hyaline (Table 12). These spores were produced in abundance.

Cleistothecial Development

The development of the sexual reproductive or perfect stage was initiated within the first 48 to 60 hours after the inoculation of the agar and resulted in a typical cleistothecium. As in the case of the pycnidia, cleistothecia were produced in abundance and, often, several stages of development could be observed in a single sector of the colony. The first cleistothecial initials appeared in a zone of the colony where the pycnidia were just reaching maturity and were produced under the surface of the agar. As the colony aged, cleistothecia seemed to arise at any level in the medium.

The cleistothecial initial was observed as a swelling of one to five cells within a submerged primary hypha (Pl. VI, Fig. 35). Morphologically it could not be distinguished from a pycnidial initial on a primary hypha. At no time

were cleistothecia observed to develop in the secondary hyphae. The position in, or on, the medium, however, did indicate which would be formed. Even so, as pointed out above, pycnidia occasionally were produced submerged in the very young cultures.

Enlargement and cell division followed in three planes. At a very early stage hypha-like structures appeared in fair abundance and were scattered over the surface of the developing mass (Pl. VI, Figs. 36-38). They branched and often fused with each other or with neighboring hyphae. The function of these branch-like structures was not definitely ascertained. It is suggested that they functioned as trichogynes because they seemed to be devoid of cell content by the time that the cleistothecium reached its maximum size, and even showed some evidence of breakdown. The cleistothecial primordium continued to enlarge by cell division into a globose structure (pl. VI, Figs. 39-43). Even at maturity the origin of the cleistothecium from a single hyphal strand was still visible (pl. VI, Fig. 42).

In four to six days after the initial appeared the structure began to darken and the outer cell walls became dark brown and distinct (Pl. VI, Fig. 43). The young cleistothecial wall consisted of two layers. The outer layer was dark brown and one cell thick. The inner layer was parenchymatous and often several cells in thickness. This inner layer gradually decreased in thickness as the

cleistothecium became older so that at maturity only the outer layer remained. The ascocarp was dark brown to black in color, more or less glabrous, globose (Pl. VI, Fig. 44), and measured 126-700 u on potato dextrose agar and 60-255 u on corn meal agar (Table 11).

The exact way in which the ascogonium arose was not determined. The cleistothecial primordium consisted of a solid mass of cells, parenchymatous in nature. Once asci began to appear the inner portion of the fructification became hollowed out, apparently by breakdown of the central cells. This breakdown progressed as the asci developed. Globose asci were produced by crozier formation (Pl. IV, Figs. 16-20) and appeared to rise in a spiral-like manner on the ascogenous hyphae. Each ascus was supported by a very short stalk-cell which may have functioned as part of the ascogenous hypha. These asci were not produced in a hymenial layer but were scattered throughout the fructification cavity by the continued growth of the ascogenous hyphae.

The asci were globose, with 32 ascospores appearing simultaneously in each ascus. When the ascospores matured the ascus wall evanesced, but the spores remained conglobate (Pl. VI, Fig. 45). When the cleistothecium was still young, but with mature spore balls, the cluster of spores did not break apart readily when pressure was applied to the slide

under the microscope. But as these spore balls aged they broke up more and more readily. Asci and spore balls measured 10.5-13.0 u on potato dextrose agar and 10.0-14.5 u on corn meal agar (Table 11).

The ascospores (Pl. VI, Fig. 46) were oblong-reniform, simple, light brown, and small, 2.0-2.9 x 2.8-5.8 u on potato dextrose agar and 2.1-2.9 x 3.5-5.2 u on corn meal agar (Table 12).

TABLE 11

SUMMARY OF MYCELIUM, CLEISTOTHECIUM, AND ASCUS AND SPORE BALL DIAMETERS ON CULTURE MEDIA[a]

	Mycelium		Cleistothecium		Ascus & spore ball	
	P.D.A.[b] u	C.M.A.[c] u	P.D.A. u	C.M.A. u	P.D.A. u	C.M.A. u
Minimum diameter	0.8	1.0	126	60	10.5	10.0
Average diameter	3.13	2.96	290	156	11.3	11.3
Medium diameter	2.95	2.95	262	150	11.0	11.0
Maximum diameter	6.1	5.5	697	255	13.0	14.5

a. Based on fifty measurements per structure on each medium
b. Potato dextrose agar
c. Corn meal agar

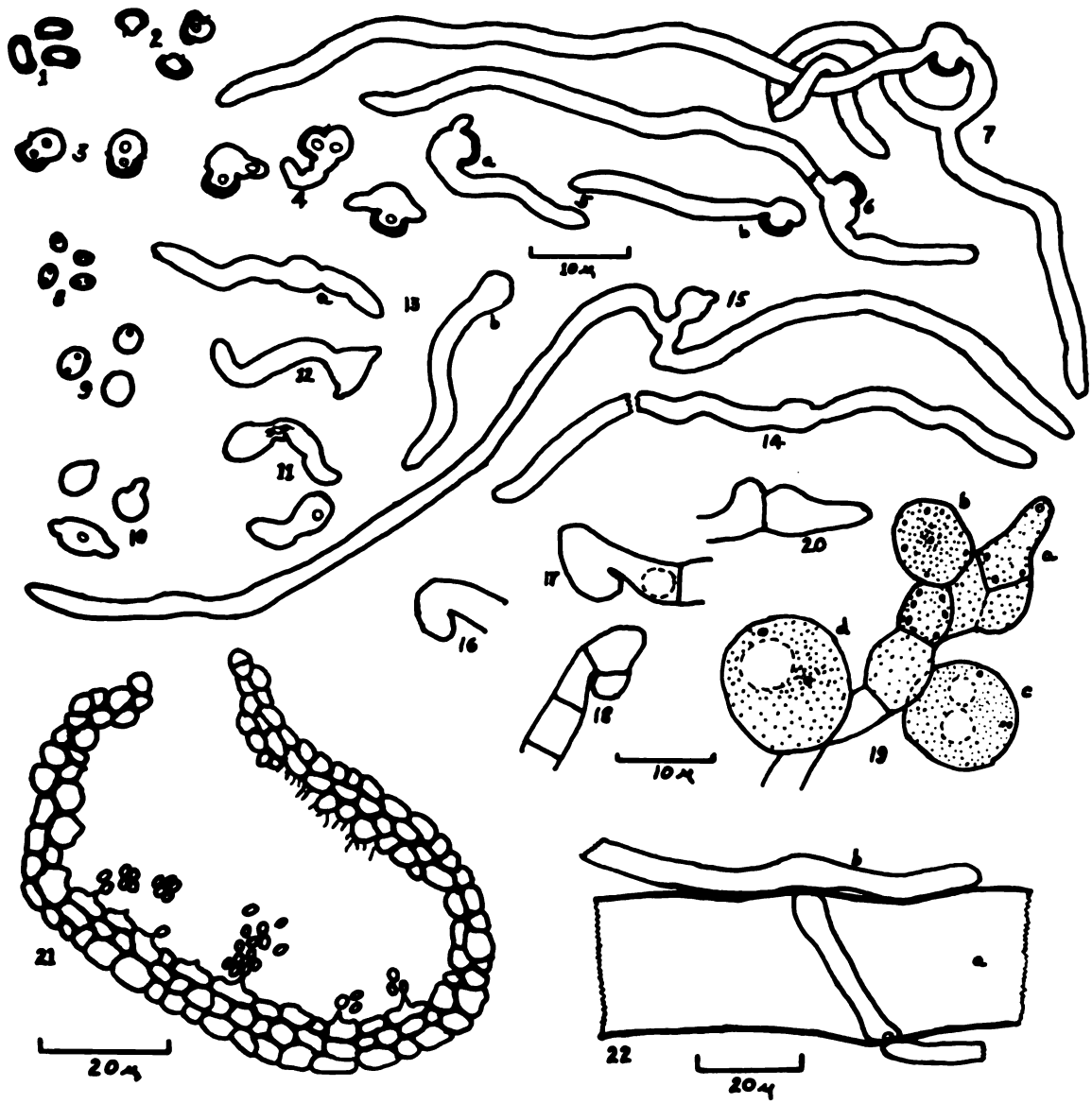
TABLE 12

SUMMARY OF PYCNIDIUM, CONIDIUM, AND ASCOSPORE LENGTH AND WIDTH ON CULTURE MEDIA[a]

	Pycnidium		Conidium		Ascospore	
	P.D.A.[b] u	C.M.A.[c] u	P.D.A. u	C.M.A. u	P.D.A. u	C.M.A. u
Minimum length	28	26	3.15	2.62	2.35	3.49
Average length	74	51	3.99	3.68	4.18	4.13
Medium length	69	48	4.05	3.73	4.32	4.14
Maximum length	164	95	4.75	4.75	5.78	5.15
Minimum width	26	26	1.51	1.78	2.03	2.11
Average width	55	36	2.38	2.30	2.51	2.46
Medium width	55	35	2.28	2.27	2.46	2.41
Maximum width	78	52	3.38	3.00	2.90	2.99

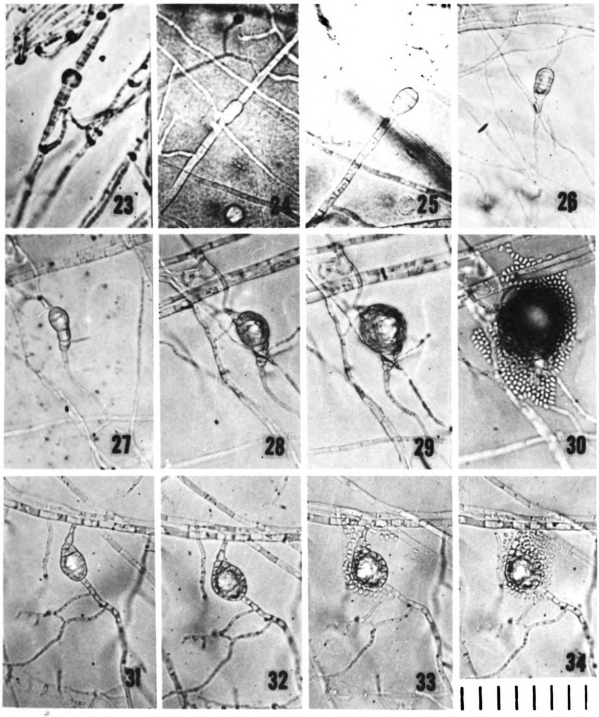
a. Based on fifty measurements per structure on each medium
b. Potato dextrose agar
c. Corn meal agar

PLATE IV



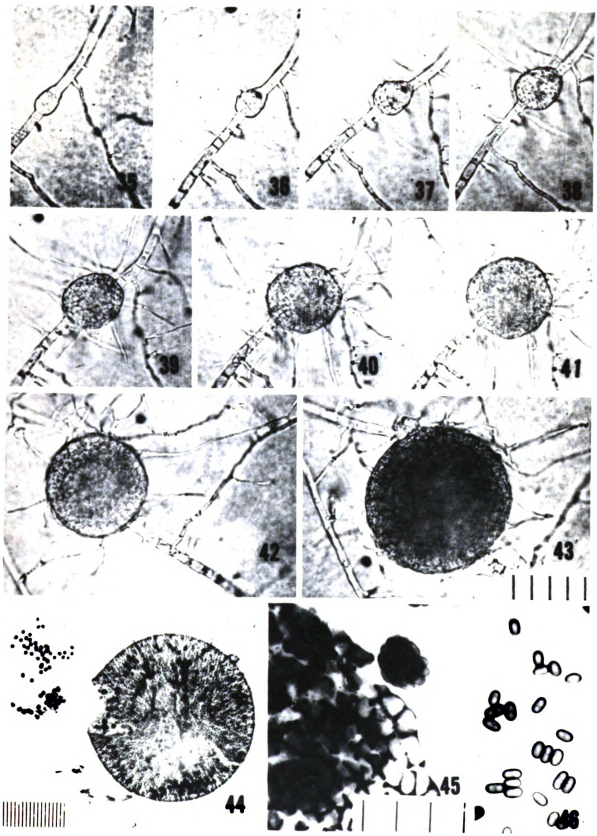
Figs. 1-7, Ascospore germination: 1) ascospores; 2) spore wall breaking open; 3) protrusion of bulbous swelling; 4) budding out of germ tubes; 5,6,7) elongation and branching of germ tube.
Figs. 8-15, Conidium germination: 8) conidia; 9) swelling of spore; 10) budding out of germ tubes; 11,12,13,14,15) elongation and branching of germ tubes.
Figs. 16-20, Ascus formation: 16,17,18) crozier formation; 19a,20) young asci; 19b,c,d) maturing asci.
Fig. 21, Cross section of a pycnidium showing conidia and conidiophores.
Fig. 22, Parenchyma cell of Aster stem penetrated by a hyphal strand: a)host cell; b) hypha.

PLATE V



Figs. 23-34, Stages in the meristogenous development of pycnidia: 23-25) one-, two-, and four-celled stages respectively on primary hyphae; 26) three-celled stage on a secondary hypha; 27-30) series of stages in the development of a single pycnidium on a secondary hypha; 31-34) series of stages in the development of another pycnidium on a secondary hypha.

PLATE VI



Figs. 35-43, Series of stages in the development of the cleistothecial primordium on a single primary hypha. Fig. 44, A crushed mature cleistothecium and scattered spore-balls. Fig. 45, Highly magnified cleistothecial wall and a spore-ball. Fig. 46, Ascospores.

DISCUSSION OF PYCNIDIOPHORA DISPERSA

Pycnidiophora has been tentatively placed in the Aspergillaceae (Eurotiaceae of many authors). Clements and Shear (11) describe the family "Eurotiaceae" as: "Mycelium abundant superficial or innate, usually saprophytic, mostly straight-walled and without hyphopodia or spines; perithecia typically on the mycelium, the wall usually parenchymic and membranous, consisting of polygonal plates as a rule, breaking up generally or at the tip when mature, ostiole present only in Micrascus, appendages present or lacking; asci typically in corymboid clusters on branched hyphae, these rarely short and approaching the umbelloid grouping, several to many, globose to clavate, few-, rarely many-spored; paraphyses regularly lacking; spores various." This is in agreement with other mycologists (7, 18). Based on this delineation, Pycnidiophora is a member of this family.

Confirmation of this placement of Pycnidiophora was made by Dr. M. P. Backus.[1] This conclusion is best expressed by quoting with permission, from a letter dated July 6, 1953 received from Dr. Backus: "I have been studying your fungus

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1. Following the experimental and morphological studies detailed in this paper, and to either confirm or refute the idea that an undescribed organism was involved, cultures were sent to Dr. Myron P. Backus, Professor of Botany, University of Wisconsin, Madison, Wisconsin.

off and on over the past two weeks and must report that I never saw anything like it and know of no genus into which it would fit. I agree with you that it must fall in the Aspergillaceae. ..... It certainly looks as if you have something new to science here, and it is all the more interesting that it is a pathogen."

It has been shown in the previous section that the fructification of P. dispersa arises from one or two adjacent hyphal cells by continued cell division. "Since this process is a true three dimensional cell division, ... the result is a true parenchymatous tissue and not a pseudo-parenchymatous matrix as in many fungi." The early development of the cleistothecial body of P. dispersa is similar to the early development of the perithecial body of Sporormia bipartis Cane as it is figured and described by Page (34). The Sporormia perithecial initial is considered by Gäumann (18) to be stromatic.

The modern tendency, stimulated by Miller (31), is to revise the classification of the Ascomycetes, particularly the Pyrenomycetes, to embrace the characters of ontogeny as well as the mature ascocarp. Gäumann (18) points out that in the Aspergillaceae sexual reproduction initiates the formation of the fructification and usually takes place by means of special copulation branches. Since the cleistothecial primordium of Pycnidiophora dispersa is considered to be stromatic, the placement of the genus in the

Aspergillaceae may be questioned. However, Gäumann says that in Penicilliopsis, a form included in the Aspergillaceae, the fructification develops vegetatively. Therefore, until a specialist of the group further defines the family, Pycnidiophora should be included in the family.

In P. dispersa the sexual process probably takes place in the stromatic-like cleistothecial primordium, but this must still be studied and worked out. It is suggested that the thin hypha-like structures that grow out from the young cleistothecial primordium function as trichogynes. Clarification of this, as well as its taxonomic position, will depend on future cytological studies of this organism.

The most unusual feature of this organism is the asexual stage. No other member of the Aspergillaceae is known to form pycnidia in the imperfect stage. The development of the pycnidium of P. dispersa was typically simple meristogenous. This development was similar to the simple meristogenous development of Phoma species as reported by Kempton (26).

Although this organism has not been described before it is possibly a common soil inhabitant that has been overlooked in the past. A fellow student[1] recently isolated a similar organism from a soil sample obtained from a lawn in the city of East Lansing.

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1. Ralph Collins, Michigan State College, East Lansing, Michigan.

Following Koch's postulates Pycnidiophora dispersa has been shown to cause damping-off of Phlox, Aster, and Lily seedlings under laboratory conditions. Further experimentation, particularly under greenhouse conditions, is desirable in order to establish the host range, the disease producing potential, and the environmental requirements of this organism.

SUMMARY

A study was conducted in which seeds of 14 ornamentals were treated with certain of ten fungicides in various combinations in a series of field and greenhouse trials.

In one or more trials significantly better seedling emergence occurred when seed protectants were used on seeds of Aster, Bachelor Button, Calendula, Cosmos, Four O'Clock, Morning Glory, Nasturtium, Phlox, Stock, Sweet Pea, and Zinnia.

Marigold was the only ornamental on which seed protectants failed to give some measure of protection against damping-off. Only limited protection was apparent on Larkspur and Dahlia seed.

Post-emergence damping-off was not controlled by the seed protectants in these experiments.

An organism, with a pycnidial imperfect stage, was isolated from a diseased Phlox seedling. This organism, by virtue of its perfect stage, was classified in the Aspergillaceae as that family is presently constituted.

This organism was shown to be pathogenic and caused damping-off.

A new genus and species, Pycnidiophora dispersa, were proposed and described to embrace this organism. The life

history and morphology were studied on corn meal agar and potato dextrose agar. Conidium and ascospore germination and hypha development were traced. Pycnidial development was found to be simple meristogenous and the cleistothecium was observed to arise in a similar manner from a few intercalary cells in a single hyphal strand. The 32-spored asci, which were scattered throughout the cleistothecial cavity, were produced by crozier formation.

LITERATURE CITED

1. Alexander, L. J., H. C. Young, and C. M. Kiger, 1931. The causes and control of damping-off of tomato seedlings. Ohio Agr. Exp. Sta. Bull. 496:1-38.

2. Atkinson, G. F., 1895. Damping-off. New York (Cornell) Agr. Exp. Sta. Bull. 94:233-272.

3. Baker, K. F., 1947. Seed transmission of Rhizoctonia solani in relation to control of seedling damping-off. Phytopath. 37:912-924.

4. Balfe, I. G., 1935. An account of sclerote-forming fungi causing disease in Matthiola, Primula, and Delphinium in Victoria. Proc. Roy. Soc. Vict. 47: 369-386.

5. Baten, W. D., 1939. Formulas for finding estimates for two and three missing plots in randomized block layouts. Mich. Agr. Exp. Sta. Tech. Bull. 165:1-16.

6. Beaumont, A., 1950. Diseases of China Asters. Gdnrs. Chron. 128:140-141.

7. Bessey, E. A., 1950. Morphology and taxonomy of fungi. Philadelphia: The Blakiston Company.

8. Bolley, H. L., 1897. New studies upon the smuts of wheat, oats, and barley, with a resume of the treatment experiments for the last three years. N. D. Agr. Exp. Sta. Bull. 27:109-164.

9. Chilton, St. J. P., D. C. Bain, and L. H. Person, 1943. Effect of seed treatments on stands of ornamental plants. (Abstract in Proc. La. Acad. Sci. 7:36).

10. Clayton, E. E., 1928. Increasing stands from vegetable seeds by seed treatment. New York (Geneva) Agr. Exp. Sta. Bull. 554:1-16.

11. Clements, F. E. and C. L. Shear, 1931. The genera of fungi. New York: H. W. Wilson Company.

12. Coons, G. H. and D. Stewart, 1927. Prevention of seedling disease of sugar beets. Phytopath. 17:259-296.

13. Dimock, A. W., 1941. The Rhizoctonia root rot of annual stocks (Matthiola incana). Phytopath. 31: 87-91.

14. Dimock, A. W., 1951. Semesan drench for Rhizoctonia control. New York State Flower Grs. Bull. 60:6-7. (Abstract, Rev. App. Mycology 30:41, 1951).

15. Doran, W. L., 1938. Germination of seeds and damping-off and growth of seedlings of ornamental plants as affected by soil treatments. Mass. Agr. Exp. Sta. Bull. 351:1-44.

16. Doran, W. L., 1946. Diseases of plants caused by soil infesting organisms, with particular attention to control measures. Annual Rep. Mass. Agr. Exp. Sta. Bull. 436:19-20.

17. Dunlap, A. A., 1936. Seedling culture in sand to prevent damping-off. Phytopath. 26:278-284.

18. Gäumann, E. A., 1952. The fungi. New York: Hafner Publishing Co. (English translation by F. L. Wynd).

19. Haenseler, C. M., 1928. Effect of organic mercury seed treatments on germination and yield of peas. N. J. Agr. Exp. Sta. Ann. Rep. 48:232-238.

20. Hartley, C., 1921. Damping-off in forest nurseries. U. S. D. A. Bull. 934:1-99.

21. Horsfall, J. G., 1930. Combating damping-off of tomatoes by seed treatment. New York (Geneva) Agr. Exp. Sta. Bull. 586:1-22.

22. Horsfall, J. G., 1938. Combating damping-off. New York (Geneva) Agr. Exp. Sta. Bull. 683:1-46.

23. Horsfall, J. G., A. G. Newhall, and C. E. F. Guterman, 1934. Dusting miscellaneous seeds with red copper oxide to combat damping-off. New York (Geneva) Agr. Exp. Sta. Bull. 643:1-39.

24. Jensen, J. L., 1888. The propagation and prevention of smut in oats and barley. Jour. Roy. Agr. Soc. England, Sec. 2, 24:397-415.

25. Kadow, K. J. and H. W. Anderson, 1937. Damping-off control: An evaluation of seed and soil treatment. Ill. Agr. Exp. Sta. Bull. 439:291-348.

26. Kempton, F. E., 1919. Origin and development of the pycnidium. Bot. Gaz. 68:233-261.

27. Lammerts, W. E., 1940. Ethyl mercury iodide - an effective fungicide and nemacide. Phytopath. 30: 334-338.

28. Leukel, R. W., 1936. The present status of seed treatment with special reference to cereals. Bot. Rev. 2:498-527.

29. Leukel, R. W., 1948. Recent development in seed treatment. Bot. Rev. 14:235-269.

30. Linnasalmi, A., 1952. Damping-off on herbaceous vegetables and ornamental plants grown under glass in Finland. Ann. (bot.-zool.) Soc. zool-bot. fenn. Vanamo, Sect. bot. 26:1-120. (Finnish summary). (Abstract, Rev. App. Mycology 32:412-413, 1953).

31. Miller, J. H., 1949. A revision of the classification of the ascomycetes with special emphasis on the pyrenomycetes. Mycologia 41:99-127.

32. Nema, K. G. and K. A. Mahmud, 1950. Damping-off of Brinjal seedlings caused by Rhizoctonia (Corticium) solani Kuhn. Mag. Agr. Coll. Nagpur 25:1-2. (Abstract, Rev. App. Mycology 30:260, 1951).

33. Newhall, A. G. and B. Lear, 1948. Soil fumigation for fungus control with methyl bromide. Phytopath. 38: 38-43.

34. Page, W. M., 1939. Contributions to the life history of certain copophilous fungi. Trans. British Mycol. Soc. 23:253-268.

35. Person, L. H. and S. J. P. Chilton, 1942. Seed and soil treatment for the control of damping-off. La. Agr. Exp. Sta. Bull. 349:1-16.

36. Prevost, I. B., 1807. Memoire sur la cause immediate de la carie ou charbon des bles et de plusieurs autres maladies des plantes. (English translation by G. W. Keitt. Phytopath. Classics 6, 1939).

37. Sharma, O. P. and K. A. Mahmud, 1950. Damping-off of Antirrhinum majus L. caused by Rhizoctonia (Corticium) solani Kuhn. Mag. Agr. Coll. Nagpur 25:47-48. (Abstract, Rev. App. Mycology 30:231-232, 1951).

38. Srivastaya, H. C., 1951. Damping-off in Hollyhocks. Sci. & Cult. 17:91-92. (Abstract, Rev. App. Mycology 31:328, 1952).

39. Srivastaya, H. C., 1951. Damping-off in Saponaria. Sci. & Cult. 17:226-227. (Abstract, Rev. App. Mycology 31:609, 1952).

40. Stoddard, E. M. and G. A. Zentmyer, 1950. Control of damping-off with 8-Quinolinol. Plant Dis. Reporter 34:236-237.

41. Stoutmeyer, V., C. Hope, and A. Close, 1941. Sphagnum for seed germination inhibits damping-off losses on unsterilized soil. Nat. Hort. Mag. 20:111-120.

42. Taubenhaus, J. J. and A. L. Burkett, 1937. The effect of chemical seed and soil treatments on damping-off and other diseases. Fiftieth Ann. Rep. Tex. Agr. Exp. Sta., 1-117.

43. Tilford, P. E., 1931. Control of damping-off of flower seedlings. Ohio Agr. Exp. Sta. Bimonthly Bull. 152:167-175.

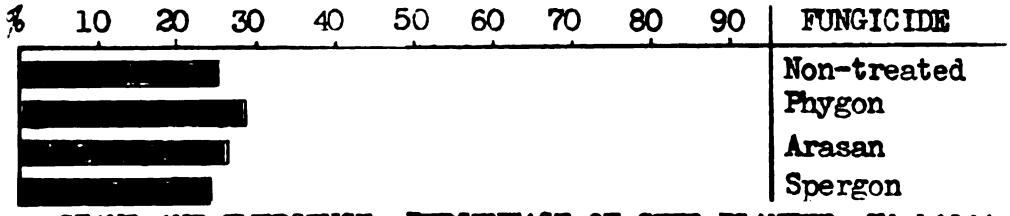
44. Tisdale, W. B., A. N. Brooks, and G. R. Townsend, 1945. Dust treatments for vegetable seed. Fla. Agr. Exp. Sta. Bull. 413:1-32.

45. Walker, J. C., 1948. Vegetable seed treatment. Bot. Rev. 14:588-601.

46. Walker, J. C., 1950. Plant pathology. New York: McGraw-Hill Book Company, Inc.

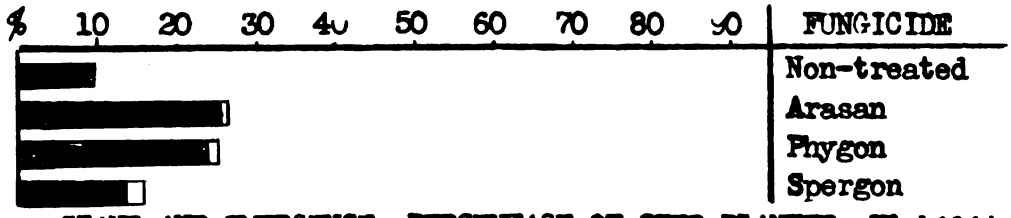
47. Wilson, J. D. and P. E. Tilford, 1933. The use of formaldehyde dust in growing seedlings. Ohio Agr. Exp. Sta. Bull. 520:1-40.

APPENDIX



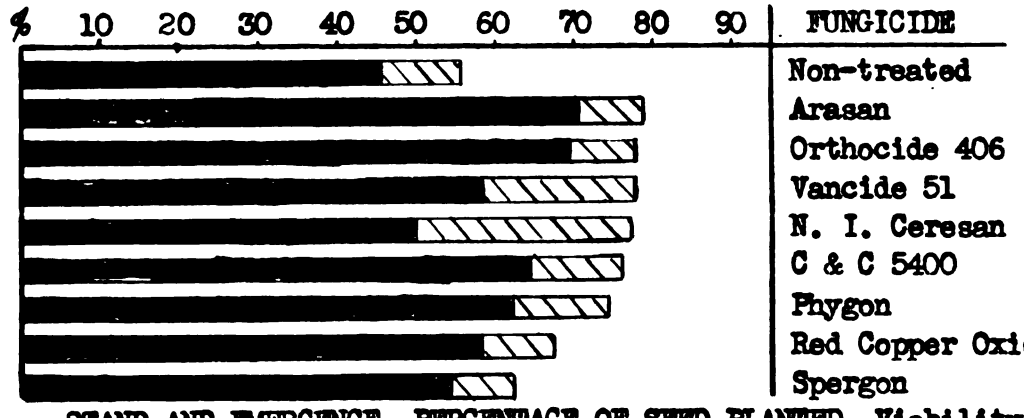
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 61%

Fig. 1. Aster (Crego), Field, 1951.



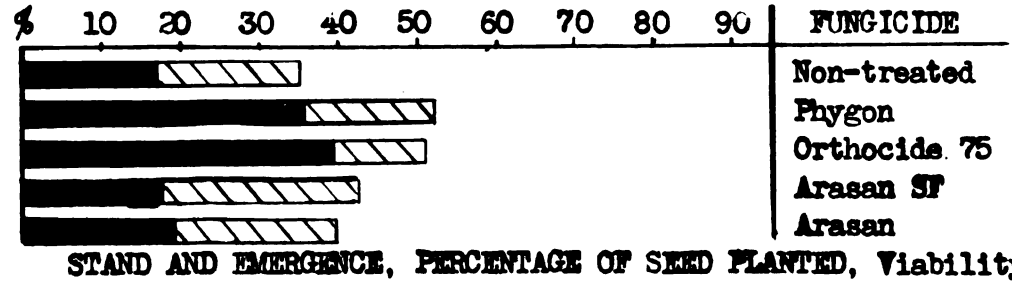
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 61%

Fig. 2. Aster (Crego), Greenhouse, December 1951.



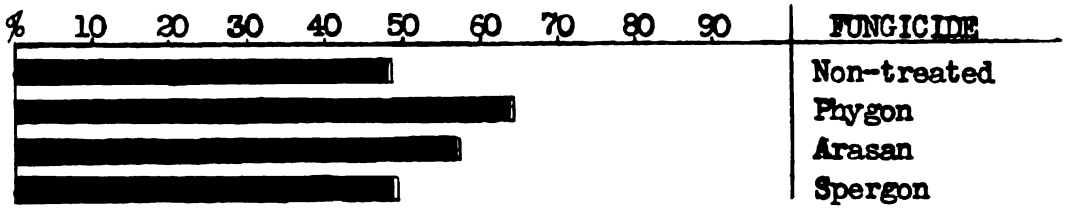
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 96%

Fig. 3. Aster (Crego), Greenhouse, March 1952



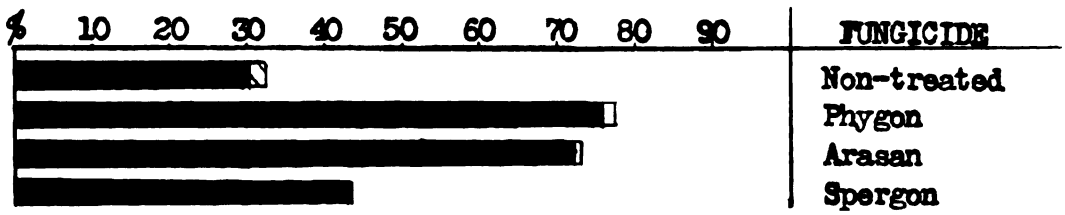
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 91%

Fig. 4. Aster (American Beauty), Field, 1952



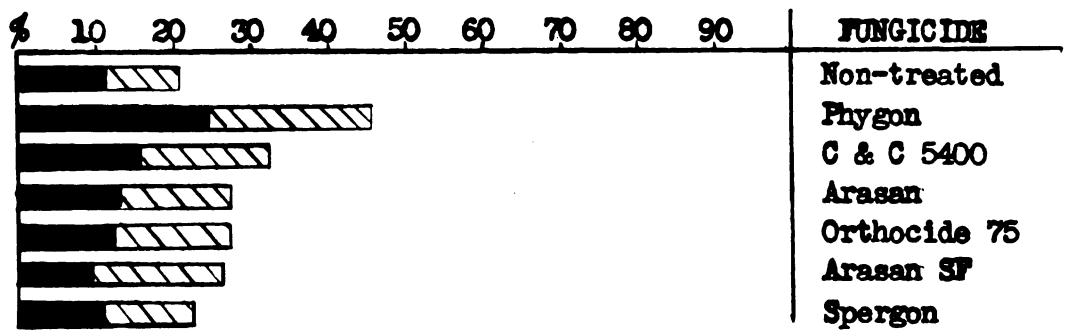
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 87%

Fig. 5. Calendula (Double Orange King), Field, 1951.



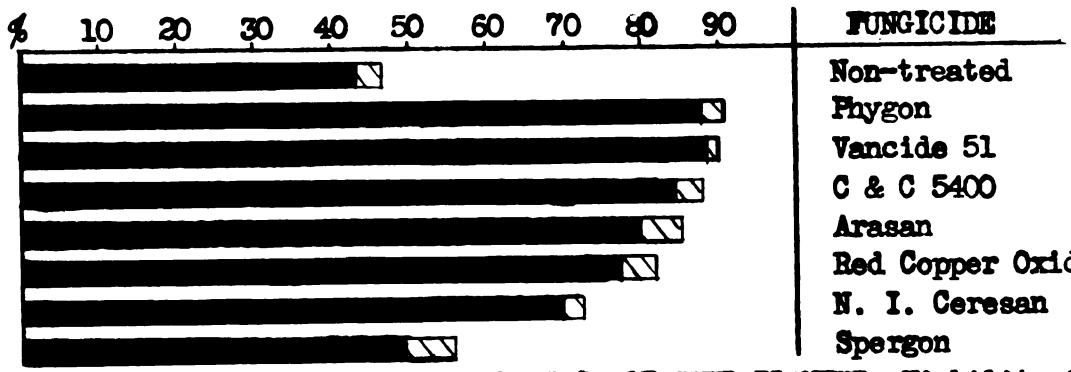
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 87%

Fig. 6. Calendula (Double Orange King), Greenhouse, December 1951.



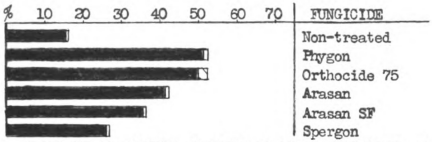
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 89%

Fig. 7. Calendula (Double Orange King), Field, 1952.



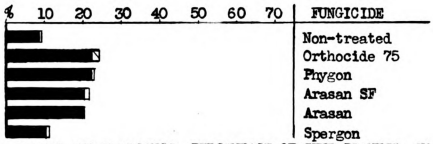
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 95%

Fig. 8. Calendula (Double Orange King), Greenhouse, January 1952.



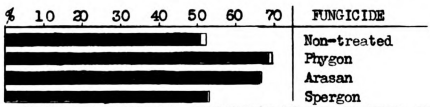
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 83%

Fig. 9. Bachelor Button, Field, July 1952.



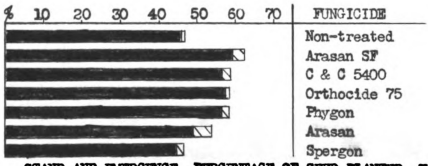
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 83%

Fig. 10. Bachelor Button, Field, September 1952.



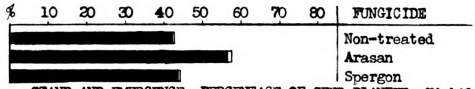
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 93%

Fig. 11. Cosmos (Sensation), Field, 1951.



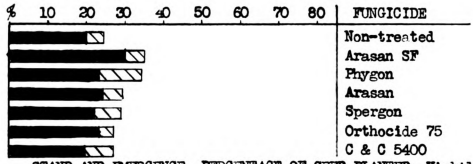
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 85%

Fig. 12. Cosmos (Sensation), Field, 1952.



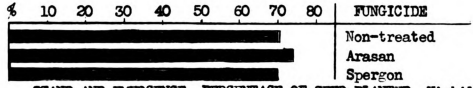
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 81%

Fig. 13. Dahlia (Unwin's Dwarf Hybrid), Field, 1951.



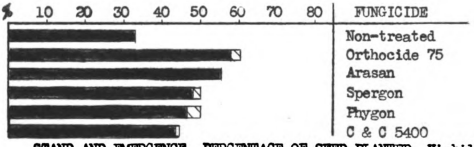
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 90%

Fig. 14. Dahlia (Unwin's Dwarf Hybrid), Field, 1952.



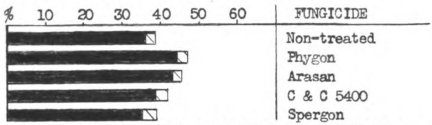
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 81%

Fig. 15. Four O'Clock (Marvel of Peru), Field, 1951.



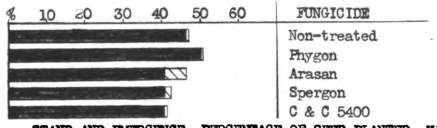
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 90%

Fig. 16. Four O'Clock (Marvel of Peru), Field, 1952.



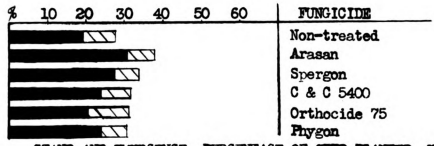
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 80%

Fig. 17. Larkspur (Giant Imperial), Field, 1951.



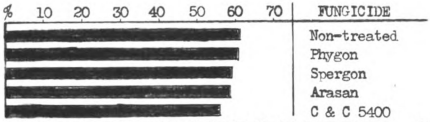
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 80%

Fig. 18. Larkspur (Giant Imperial), Greenhouse, December 1951.



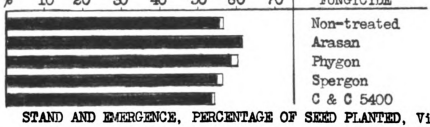
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 84%

Fig. 19. Larkspur (Double Mixed), Field, 1952.



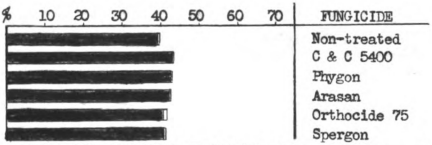
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 74%

Fig. 20. Marigold (Harmony Dwarf), Field, 1951.



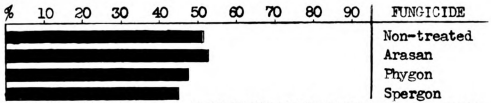
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 84%

Fig. 21. Marigold (Harmony Dwarf), Greenhouse, December 1951.



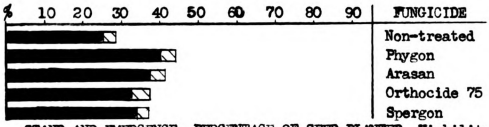
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 87%

Fig. 22. Marigold (African), Field, 1952.



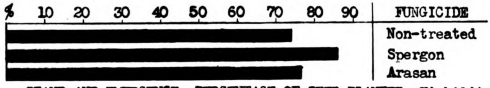
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 61%

Fig. 23. Morning Glory (Heavenly Blue), Field, 1951.



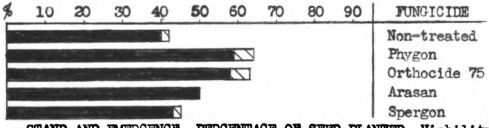
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 56%

Fig. 24. Morning Glory (Heavenly Blue), Field, 1952.



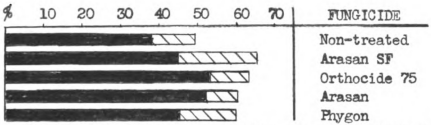
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 82%

Fig. 25. Nasturtium (Dwarf Choice), Field, 1951.



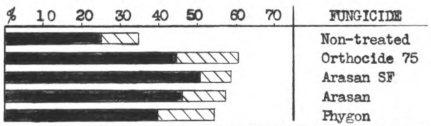
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 60%

Fig. 26. Nasturtium (Glor. Gleam Hybrid), Field, 1952.



STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 92%

Fig. 27. Zinnia (Giant of California), Field, June 1952.



STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 92%

Fig. 28. Zinnia (Giant of California), Field, September 1952.

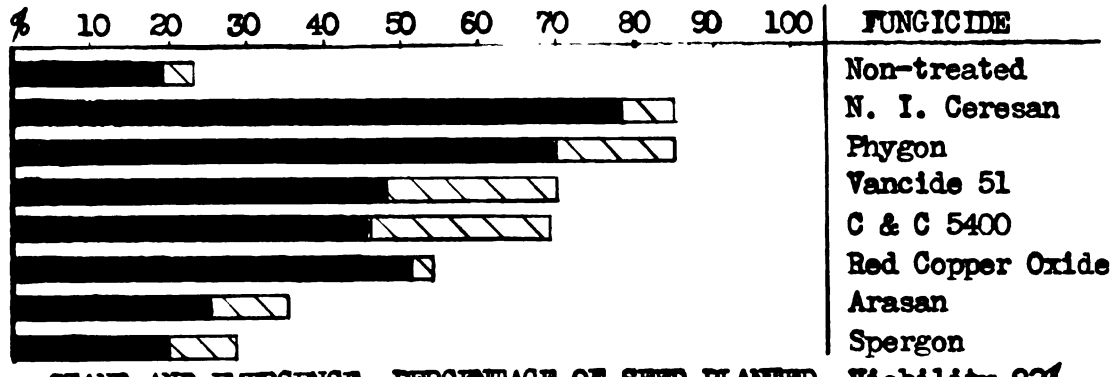


Fig. 29. Sweet Pea, Greenhouse, January 1952.

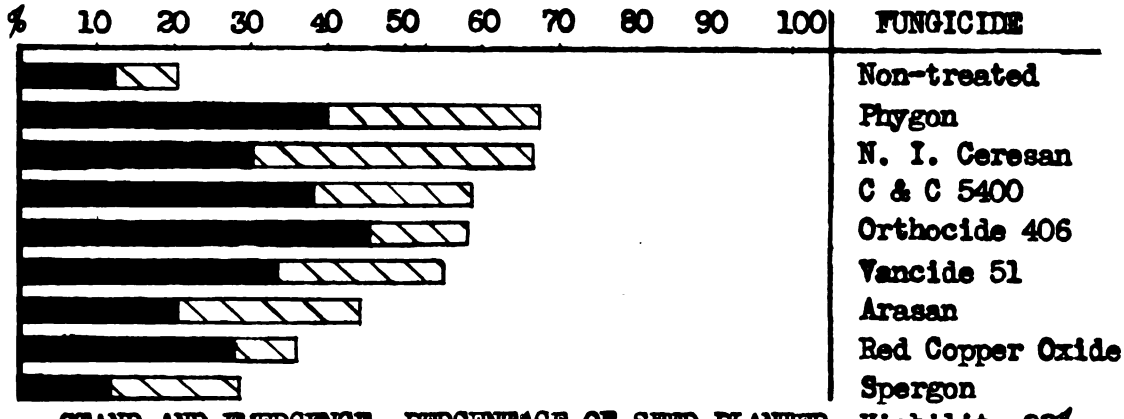


Fig. 30. Sweet Pea, Greenhouse, February 1952.

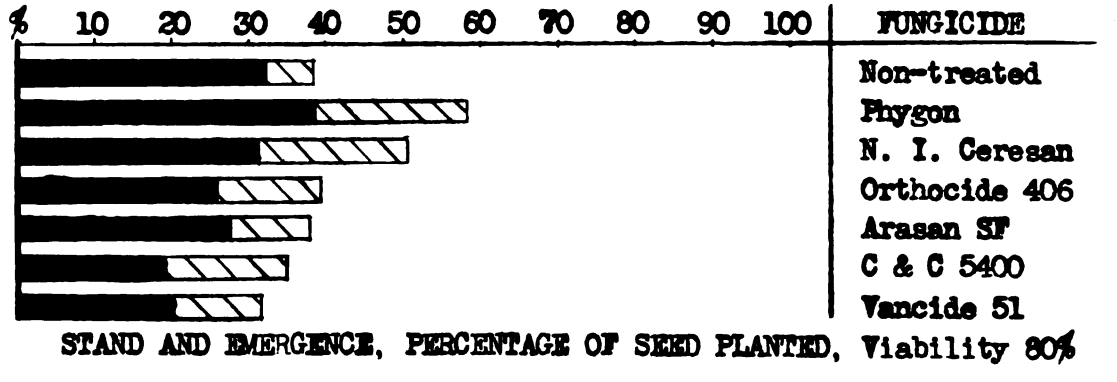
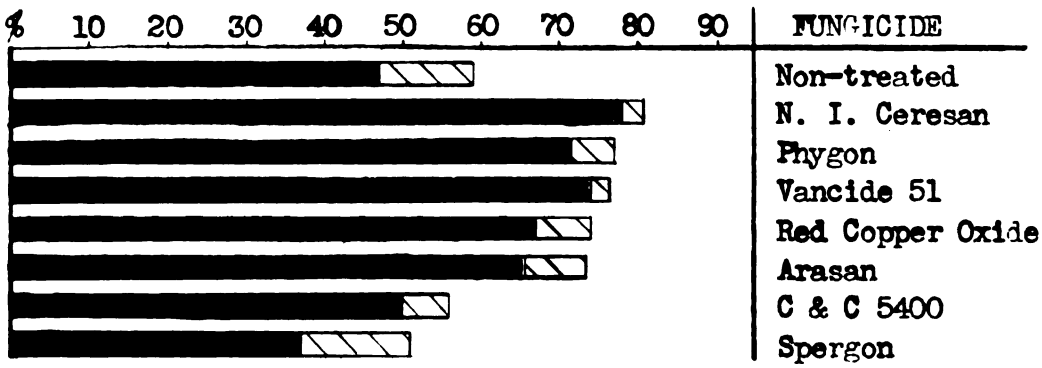
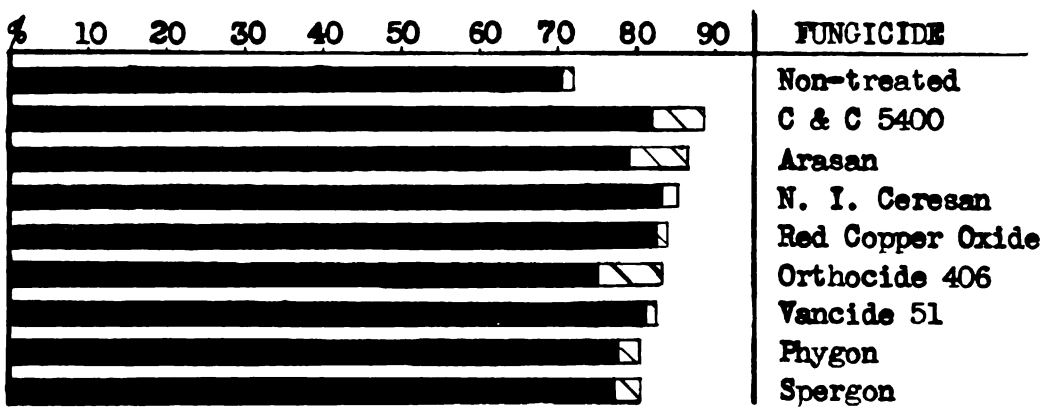


Fig. 31. Sweet Pea, Field, 1952.



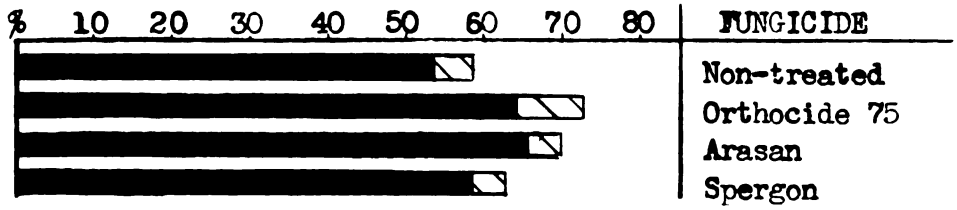
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 93%

Fig. 32. Stock, Greenhouse, January 1952.



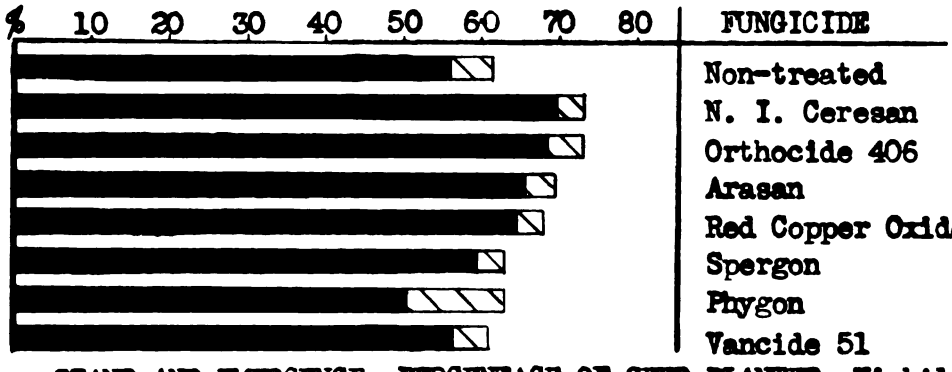
STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 93%

Fig. 33. Stock, Greenhouse, February 1952.



STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 85%

Fig. 34. Phlox (Drummondii), Greenhouse, Decxmber 1952.



STAND AND EMERGENCE, PERCENTAGE OF SEED PLANTED, Viability 85%

Fig. 35. Phlox (Drummondii), Greenhouse, January 1953.