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RELATIVE VALUE AND MODE OF ACTION OF SOME FUNGICIDES USED AS SEED DISINFECTANTS AND PROTECTANTS

WITH A SUMMARY IN DUTCH

RELATIEVE WERKZAAMHEID VAN ENIGE FUNGICIDEN, ALS ZAADONTSMETTERS EN ZAADBESCHERMERS TOEGEPAST

by *(door)*

AHMED SOLIMAN SAMRA [1])

(Laboratorium voor Phytopathologie, Landbouwhogeschool, Wageningen. Mededeling no. 162)

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[1]) Plant pathologist at the Plant Disease Section, Ministry of Agriculture, Caïro, Egypt

GENERAL INTRODUCTION

Seed treatment as a method for the control of plant diseases has been practised since the accidental discovery, some 300 years ago in England, that sea water would inhibit the development of smut of wheat. A further stage of the development of chemical seed treatment occurred in 1761, when SCHULTHUSS (71) suggested the use of copper sulphate solution in place of salt water.

The scientific foundation of chemical seed treatment, however, was laid by PREVOST (62), who in 1807 discovered the parasitical nature of the "smut" disease, against which copper sulphate had been used with success.

From 1860 to '95 the search for a suitable substitute in stead of copper sulphate, which was found to have an objectionable action upon the seed, continued. The most promising of the materials tried appears to have been salicylic acid, suggested by SCHRÖDER (70) in 1892, which, however, has the disadvantage of being too expensive for general use.

KELLERMAN & SWINGLE (42) in 1891 found a satisfactory seed disinfectant in liver of sulphur (potassium polysulphide). Sulphur has afterwards been found to be of great value as a seed dressing to be recommended where a cheap, simple and safe method is essential. In 1895 the new disinfectant formaldehyde was introduced for the treatment of cereals. For some time it became one of the most popular chemicals for seed treatment. About 1890 a new field was opened by BOLLEY (5), who for the control of potato skin diseases immersed the "seed" in mercuric chloride. At the beginning of the twentieth century HILTNER (35) introduced this compound for the control of cereal diseases by seed treatment. He found it valuable for the control of Fusarium infection of rye.

A number of commercial preparations (e.g. Fusariol, Fusafine), in which mercuric chloride was incorporated, were put on the market. The significance of HILTNER'S discovery lies in the fact that the Fusarium disease of rye is not transferred by spores on the exterior of the seed, but by a dormant mycelium

within the seed. It is well known that the fungicide must remain on the seed in order to prevent infection of the coleoptile.

Hiltner's observation thus indicated the possibility of utilizing a protective fungicide for the control of diseases transferred within the seed.

In 1914 the first record of the use of organic mercury compounds was brought by RIEHM (67), who reported "chlorophenol mercury" to be successful for combating bunt infection of wheat seed. Uspulun was the first organic mercury fungicide, marketed in 1915 by the firm of Bayer in Germany. This product was still used for an immersion treatment.

Dusts were introduced by DARNELL-SMITH (12) about the time of the first worldwar.

RIEHM (1920 and 1923) (68 and 69) and other German workers as GASSNER (1923) (22) continued their work on organo-mercurials and it was not long before a general review could be published including organo-mercurials used as dusts. In this review it was already appreciated that these materials controlled a whole range of diseases, several cereal smuts, leaf stripe diseases (*Pyrenophora, Helminthosporium*) and also foot rots (*Fusarium* and allied diseases) according to GRAM 1926 (24). In 1929 CLAYTON (9) wrote, that a short immersion ("instant dip" treatment) of the seed in suspensions or solutions of organic mercury compounds is more satisfactory than the mercuric bichloride treatment. The use of organo-mercury compounds for seed treatment was extensively examined by BRETT, DILLON WESTON & BOER (6) and many others.

In 1938 MARSH (52) discussed the fungicidal properties of tetramethylthiuramdisulfide. This organic sulphur compound had been described already in 1934 by THISDALE & WILLIAMS (75). It is now commonly indicated as thiram or TMTD, and has been the subject of much work concerning seed treatment. In 1944 already it had come to general use in America (arasan, thiosan) and England (sulsol) as a dust treatment of seeds.

In later years these organic materials came into use as a slurry treatment, which means that the seed was mixed with a small amount of highly concentrated suspension of the fungicide, so that the necessity of drying the seed after the treatment was avoided.

During the last few years "antibiotics" received increasing attention from plant pathologists for the control of plant diseases. The term has come into use for products of living organisms that may be used for combating disease. Recently antibiotics have been studied in relation with seed infections, for which they apparently offer advantages in certain cases.

The purpose of this study was to investigate the mode of action of some fungicides applied to seed and to soil: a. to combate seed-borne infections and b. to protect seed and seedling after sowing against invasion by omnipresent, more or less pathogenic soil-borne organisms.

In the present publication attention is given to both these subjects. In part I a case is studied, in which only the disinfection of the seed against seed-borne fungi is of importance; in part II a case is studied, in which the protection is of exclusive importance.

The study is restricted to chemical seed treatment. The possibilities of the use of fungicides for seed treatment as well as the characteristics of their action were studied. This has been done by a suitable choice of two subjects viz.: mercurial treatment of radish seed and thiram treatment of corn seed is justified in the introductory sections of the two parts of the publication.

PART I

THE PRESENCE OF *ALTERNARIA SPP.* IN RADISH SEED AND ITS CONTROL BY CHEMICAL TREATMENT

Notwithstanding a development of nearly two centuries the present knowledge of chemical seed treatment is far from complete. In many different crops there are many seed-borne diseases which cannot yet effectively be combated by chemical treatment of the seed. For certain of these seed diseases other methods of control have been developed, such as heat treatment.

Internal seed infections may be effectively destroyed by a hot-water treatment. However, this method is not suited for every kind of seed, it has practical disadvantages, and, moreover, it is outside the scope of this work.

The first subject of this study was to find more details concerning the chemical disinfection of seeds with more or less deep-seated infections, to compare fungicides of different types in their action. Also the location of the infection has to be studied. It was not easy to find a suitable kind of seed for this investigation.

Most seed-transmitted infections are either largely superficial or quite internal, and consequently either easy or impossible to destroy by chemical means. A kind of seed was required with a high percentage of infection.

After ample consideration radish seed with Alternaria infection was chosen for the purpose, although radish is not an important crop and the seed transmission of the disease is relatively unimportant. However, radish seed with Alternaria infection, mainly *Alternaria raphani* GROVES & SKOLKO is easily to obtain in the Netherlands and the infection is not effectively destroyed by the common chemical seed treatments. Probably it is located partly in the outer layers of the seed coat, partly in deeper tissues, but this has not been studied in detail. It is easily detected in a germination test in filter paper medium. These reasons were thought to counterbalance the disadvantages of the choice.

In relation with fungicide treatment of seeds we may distinguish, roughly speaking, between two actions. In the first place the fungicide may be used for combating seed-borne infections, and for this purpose it may be indicated as a disinfectant; in the second place it may be used for protecting the seed and seedling after sowing against invasion by soil-borne micro-organisms, and in this sense we may speak of a protective action. Now as the first subject of study radish seed was chosen, with its seed-borne Alternaria infection, because here we have a case in which only seed-borne infections play a role, and consequently only disinfection by the fungicide is necessary.

CHAPTER I

RELATIVE EFFICIENCY OF DIFFERENT FUNGICIDES AGAINST ALTERNARIA SEEDLING BLIGHT OF RADISH

1. INTRODUCTION

Chemical seed treatments reported so far for the control of *Alternaria spp.* in radish have proved of restricted value. GROVES & SKOLKO (28) found that,

when infected seed was plated on agar after treatment with ceresan, semesan, arasan or spergon, the percentage of *Alternaria raphani* (GR. & SK) which developed was only slightly reduced. Consequently they stressed the necessity of using healthy seed. In the plant pathology section of the Rept. Dept. Agric. Canada (61), a greenhouse test is described, in which the application of arasan dust to the seed, increased the emergence of a sample of radish seed with 58 % percent infection from 59.4 % to 80 %. TAYLOR *et al.* (74) reported, that the seedling emergence of radish may be improved by treating the seed with an organomercury disinfectant.

MCLEAN (56) on the other hand reported, that although a hotwater treatment of 25 minutes at 50°C killed the pathogen in the seed, this procedure failed to increase germination. In another Canadian publication it is indicated, that seed treatment with 1 % ceresan may reduce the seedling blight stage of the black pod-blotch fungus (*A. raphani*) in greenhouse experiments with 25 to 40 % (64). However, the most effective control of *A. raphani* in radish seed was obtained by soaking in water of 50°C for 10 to 40 minutes. The best chemical control was achieved with semesan Jr and puratized N5E. In greenhouse and field experiments of the Dutch Seed Testing Station it was observed that seed treatment with different fungicides resulted in healthier seedlings, but rarely in a higher percentage of emergence.

Finally ATKINSON (4) reported that appreciable increase in emergence and decrease in seedling infection by *A. raphani* was effected by seed treatments with some of the common fungicidal dusts.

2. MATERIALS AND METHODS

In the following experiments about 17 different seed disinfectants were tested for their efficiency in controlling *Alternaria spp.* in radish seed. The testing was carried out in blotter paper medium.

In order to give volatile disinfectants the opportunity for full activity, the seed was normally treated two to three days in advance of the testing.

The fungicides were applied in closed glass jars. The dusts were carefully weighed on a sensitive balance; the fluid treatments were measured by means of a pipette. After tightly screwing on the lid the jars were shaken immediately to distribute the chemicals.

For testing the seed, metal trays of about 10 × 25 cm, with a perforated bottom, were used. The bottom was covered with two layers of moist filter paper, of which the upper one was provided with a hundred holes in which to place the round seeds. The trays, with 100 seeds each, were incubated for seven days in a 20°C germinator of high humidity. After this week the Alternaria symptoms could be observed clearly on the seedlings and on the non-germinated seeds. Slightly attacked, non-treated seeds gave rise to seedlings with black spots and points on the cotyledons and dark streaks on the hypocotyl. In case of severe seed infection, germination may result in a malformed little seedling with severe signs of decay and/or covered with the typical olive-greyish mycelium. Severe infection also may totally inhibit germination; in this case the seeds themselves are covered with the greyish-green mycelium with spores.

In the beginning it was difficult to distinguish between the symptoms of Alternaria infection and those of threshing injury, which is also very prevalent

in Dutch-grown seed. After gaining some experience, however, it was possible to recognize the two as such. The Alternaria spots on the cotyledons are black to dark brown, with a sharp border, and often so small that they are scarcely or not visible with the naked eye; the stripes on the hypocotyl are narrow, often about one mm long, dark brown to black; in addition the base of the stem may show a brown, soft, water-soaked area, which afterwards causes the plant to bend over and die. The threshing injury is the cause of the spots on the cotyledons which are more greyish and with a vague borderline; in addition the growing point may be damaged, and the hypocotyl may show curvatures. For our purpose it is only important to learn to distinguish between the two kinds of spots. In the course of the work different seed lots were used. This has the advantage, that the conclusions are not based on the behaviour of a single seed lot, which might be somewhat peculiar in its properties. It has the disadvantage that the figures obtained in the different experiments are not directly comparable.

The choice of the substances used was rather arbitrary, but at least some dry and wet mercurial treatments and thiram (TMTD) compounds were included. In further work the interest was centered on some of the more important types of fungicides.

The fungicides used in the first experiments are listed in table 1.

TABLE 1. Materials used, their chemical composition and name of supplying firm.

Material	Chemical composition	Firm
ceresan-new	methoxyethyl mercury chloride	N.V. Agrochemie
ceresan-wet	methoxyethyl mercury silicate	N.V. Agrochemie
germisan-dry	org. Hg compound (1.7% Hg)	Landbouwb. Wiersum
germisan-wet	org. Hg compound (2.6% Hg)	Landbouwb. Wiersum
panogen	(methylmercure) quanidine	G. Ligtermoet & Zn.
aagrano-48	org. Hg compound (1.3% Hg)	Landbouwb. Wiersum
V.N. 15	org. Hg verb. (0.9% Hg)	Landbouwb. Wiersum
no. 9815	org. Hg verb. (1.7% Hg)	Landbouwb. Wiersum
M.A. 101	org. Hg verb. (0.6% Hg)	Landbouwb. Wiersum
aagusan	org. Hg compound (0.45% Hg)	Landbouwb. Wiersum
aamertam	50% TMTD + org. Hg compound (1.25% Hg)	Landbouwb. Wiersum
aapirol 80	80% TMTD spray-powder	Landbouwb. Wiersum
aatiram	50% TMTD powder	S. Ligtermoet & Zn.
liro-thiram		N.V. Landbouwchemie
arasan SF-X	75% TMTD	N.V. Agrochemie
copper-Bayer	Coperopychloride	Kon. Zoutindustrie
coneprox	Coperopychloride	Boekelo – Hengelo

3. EXPERIMENTS AND RESULTS

The results of a number of experiments with the fungicides mentioned above are summarized in table 2. Because different fungicides were included in different experiments taken with different seed samples and each with their own controls, the figures indicated for the different fungicides in this table are not directly comparable. They have to be expressed in relation to the checks belonging to each of them.

In table 2 the columns from I to V indicate respectively, for each fungicide the total number of seeds used, the number of healthy seedlings, the number of

dead disease-free seeds, diseased seedlings + diseased non-germinated seeds, and the percentage of disease as calculated from these figures.

For each fungicide at least 200 seeds were used, but for the more interesting compounds greater numbers of seeds, up to 1200, were used. Column VI indicates the average percentage of infection in the controls belonging to the experiments in which these fungicides were included. Column VII gives the reduction in disease as calculated from column V and VI.

TABLE 2. Relative efficiency of different fungicides in controlling seedling blight of radish

Fungicide and dosage	Number of seeds and seedlings				Percentage of infection	% of infection in controls	% of reduction in disease
	Number of seeds used I	Healthy seedlings II	Dead but healthy seeds III	Diseased seedlings and non-germ. seeds IV	V	VI	VII
ceresan-wet 2%, 5 min.	200	161	38	1	0.5	46	99.0
ceresan-wet 2%, dipped	200	161	36	3	1.5	46	96.9
germisan-wet 2%, 5 min.	200	159	38	3	1.5	46	96.9
germisan-wet 2%, dipped	200	160	37	3	1.5	46	96.9
germisan-dry 0.3%	200	156	34	10	5.0	46	89.1
aagrano-48y dry, 0.3%	200	156	32	12	6.0	46	87.0
aamertam dry, 0.3%	200	159	28	13	6.5	46	85.8
no. 9815 liquid 0.3%	200	150	36	14	7.0	46	84.8
ceresan-new dry, 0.3%	1200	1007	108	85	7.0	31.6	77.8
V.N. 15 liquid, 0.3%	200	142	37	21	10.5	46.0	77.1
M.A. 101 liquid, 0.3%	200	139	40	21	10.5	46.0	77.1
panogen liquid, 0.3%	400	320	52	30	7.5	32.25	76.7
aatiram dry, 0.3%	200	145	28	27	13.5	46.0	70.6
aapirol dry, 0.3%	1100	887	106	107	9.7	31.1	68.6
aagusan dry, 0.3%	600	455	26	119	19.8	27.7	28.5
copper-Bayer dry, 0.3%	400	248	13	139	34.7	32.3	0
coneprox dry, 0.3%	400	253	15	132	33.0	32.3	0

A study of table 2 will show that the organic mercury compounds were the most effective disinfectants used. The tetramethylthiuramdisulfides came in the second place in reducing seedling blight of radish as caused by *Alternaria spp.* The copper compounds had no effect whatever in reducing the disease. The short immersion in solutions of ceresan-wet or germisan-wet were, however, still more effective than the mercurial dusts. The best mercurial dust – ceresan dry – gave about 84 % reduction in disease, whereas ceresan-wet decreased the infection by about 99 %.

Ceresan-wet and germisan-wet were the only materials tried as wet or dip treatment and at the same time were the most effective treatment used. This is in accordance with HARVE'S results (7).

This worker supposed that the pathogens within the seed treated with a fungicide dust would still be protected more or less by the dry cuticle. As the results of a wet treatment the cuticle swells, becomes soft and probably more permeable, allowing better penetration of the chemicals.

This suggests that the thiram compounds might also be more effective when administered in the form of solutions or suspensions. To test this "tripomol", a wettable thiram preparation was tried in suspension as well as a dust treatment.

TABLE 3. Treatments with tripomol-80 suspensions in water (200 seeds for each treatment)

Fungicide and dosage	Seedlings		Non-germ. seed		% of infection	Reduction of infection
	healthy	diseased	infected	non-infected		
tripomol-susp. 2%, 2 min.	128	16	11	45	13.5	76.0
tripomol-susp. 2%, 4 min.	121	15	13	51	14.0	75.0
tripomol-susp. 4%, 2 min.	132	13	8	47	10.5	81.2
tripomol-susp. 4%, 4 min	133	17	9	41	13 0	76.8
tripomol 80 dry	141	11	9	39	10.0	82.1
control	54	86	26	34	56.0	–

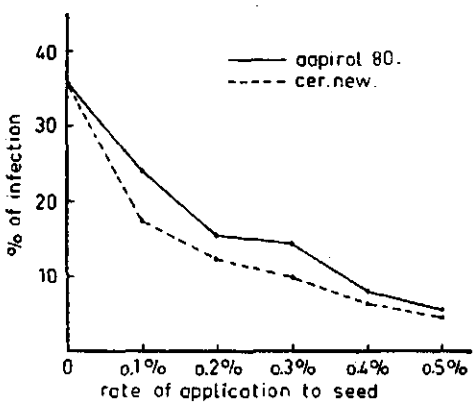
These results indicate that the dip treatments with the different tripomol suspensions are not more effective than the dust treatment with the same compound.

Consequently Harvey's hypothesis does not hold under the circumstances of our experiment.

In the following experiment the relative efficiency of different dosages of dry treatments with ceresan-new and aapirol-80 in controlling *Alternaria spp.* in radish seed has been studied.

TABLE 4. Percentage of infection obtained by using different dosages of dry treatment with ceresan-new and aapirol-80

Fungicide and dosage	Seedlings		Non-germ.seeds		% of infection
	healthy	diseased	infected	non-infected	
0.1% cer.new	146	22	9	23	15.5
0.2% cer.new	150	21	4	25	12.5
0.3% cer.new	155	19	1	25	10.0
0.4% cer.new	163	13	0	24	6.5
0.5% cer.new	175	7	2	16	4.5
0.1% aap.-80	138	34	14	14	24.0
0.2% aap.-80	140	26	5	29	15.5
0.3% aap.-80	158	26	3	13	14.5
0.4% aap.-80	169	11	5	15	8.0
0.5% aap.-80	171	9	2	18	5.5
control	117	52	20	11	36.0



GRAPH 1. Relative efficiency of using different dosages of dry treatment with ceresan-new and aapirol-80.

The results shown in table 4 and graph 1 indicate, that the percentage of infection decreases when the dosage of ceresan-new or aapirol is increased.

When used in high dosages both fungicides give nearly the same amount of control of the infection.

However, both treatments fail to result in complete control.

In the next experiments a wet treatment in several concentrations and durations is included and compared with the dry treatments in different dosages.

TABLE 5. Percentage of infection obtained by using several concentrations and dosage of wet and dry treatments

Fungicide and dosage	Seedlings		Non-germ. seeds		% of infection
	healthy	diseased	infected	non-infected	
cer.wet 2.5%; 5 min. .	204	1	0	95	0.3
cer.wet 5.0%; 5 min. .	163	0	0	137	0
cer.wet 10.0%; 5 min. .	71	0	0	229	0
cer.new 0.25%	205	37	2	56	13.0
cer.new 0.5 %	227	16	1	56	5.6
cer.new 1.0 %	236	9	0	55	3.0
aap.-80 0.25%	218	25	5	52	10.0
aap.-80 0.5%	222	17	5	56	7.3
aap.-80 1.0%	237	9	1	53	3.3
control	81	129	38	52	55.6

Ceresan-wet is the best of the fungicides used but it has a toxic effect on the seeds when administered in high concentrations. The percentage of infection decreases when the dosage of the dry fungicides is raised. Both of the dust fungicides fail to effect complete control of the disease, even when used in dosages up to 1 %.

CHAPTER II

THE USE OF WET TREATMENT WITH MERCURIALS AGAINST ALTERNARIA INFECTION OF RADISH SEED

1. PHYTOTOXICITY OF CERESAN-WET AND GERMISAN-WET

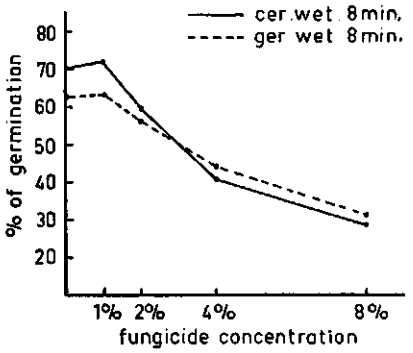
It was noticed in the preceding experiment that the stand in some blotter tests was poor as the result of treatment with too high concentrations of the mercurial solutions. This suggested that ceresan-wet and germisan-wet have a deleterious effect on the viability of the radish seed. To check this point a sample of radish seed without Alternaria infection was treated with the fungicides mentioned. Untreated seed was germinated as a control. The fungicides were also given in higher concentrations so as to obtain as much information as possible on the toxicity for the seed; 200 seeds were used for each treatment.

From table 6 and graph 2 the following conclusions may be drawn.

A concentration of 1 % of the fungicides, used 1 to 8 minutes, has no delete-

TABLE 6. Influence of overdosage of mercurials on percentage of germination of radish seed

Fungicide and concentration	Time of dipping			
	1 min.	2 min.	4 min.	8 min.
1% cer.wet	64	64	62	63
2% cer.wet	60	52	54	57
4% cer.wet	59	55	50	44
8% cer.wet	47	43	29	32
control 63.0%				
1% germ.wet	71	72	69	72
2% germ.wet	59	53	57	60
4% germ.wet	58	61	54	41
8% germ.wet	34	30	26	29
control 70.0%				



GRAPH 2. Influence of overdosage of mercurials on percentage of germination of radish seed.

rious effect on the viability of radish seed. The time of treatment both for ceresan-wet and germisan-wet, seems to be of little importance for the viability of the seed in comparison with the concentration of the fungicides.

2. EFFICIENCY OF CERESAN-WET AGAINST *ALTERNARIA SPP.* IN RADISH SEED

In order to obtain more data on the wet treatment with ceresan, in this section a study has been made on concentration and duration resulting in better control of the fungus in radish seed without having a deleterious effect on seed viability. The seed was kept from 1 to 8 minutes in the stronger solutions and up to 2 hours in the lower concentrations. For testing the 1.5 and 1.0% solutions 200 seeds were used; for the lower concentrations 100 seeds were used.

The seedlings after treatment with 0.5% ceresan-wet during 1 and 2 hours, were shorter and thicker than usual. This probably is due to the toxic effect of these treatments. The disease symptoms appearing after treating with 1.5 and 1% ceresan-wet, were only very slight, being just visible with the naked eye as little black spots on the cotyledons.

The results of these experiments are:

1. Using a solution of 1.0% ceresan-wet during 4 to 8 minutes proved to be

TABLE 7. Efficiency of different durations and concentrations of ceresan-wet in controlling *Alternaria spp.* in radish seed

Fungicide treatment	Seedlings		Non-germ. seed		Abnorm. seedlings	% of infection
	healthy	diseased	infected	non-infected		
cer. 1.5% moment . .	125	6	1	48	20	3.5
cer. 1.5% 1 min. . . .	139	2	0	42	17	1.0
cer. 1.5% 2 min. . . .	122	1	0	56	21	0.5
cer. 1.5% 4 min. . . .	128	0	0	47	25	0
cer. 1.5% 8 min. . . .	125	1	0	50	24	0.5
cer. 1.0% moment . .	132	14	0	34	20	7.0
cer. 1.0% 1 min. . . .	134	8	0	44	14	4.0
cer. 1.0% 2 min. . . .	144	4	0	40	12	2.0
cer. 1.0% 4 min. . . .	142	0	0	40	18	0
cer. 1.0% 8 min. . . .	138	0	0	44	18	0
control	50	82	37	24	7	59.5
cer. 0.5% 7½ min. . . .	58	4	0	17	21	4
cer. 0.5% 15 min. . . .	56	0	0	20	24	0
cer. 0.5% 30 min. . . .	56	0	2	20	22	2
cer. 0.5% 1 hour . . .	51	0	0	20	29	0
cer. 0.5% 2 hours . .	48	0	0	17	35	0
cer. 0.25% 7½ min. . .	51	1	4	20	15	14
cer. 0.25% 15 min. . .	46	5	0	22	27	5
cer. 0.25% 30 min. . .	56	5	0	20	19	5
cer. 0.25% 1 hour . .	57	4	1	23	15	5
cer. 0.25% 2 hours . .	53	1	0	26	20	1
cer. 0.125% 7½ min. . .	54	7	8	17	14	15
cer. 0.125% 15 min. . .	44	11	6	24	15	17
cer. 0.125% 30 min. . .	46	6	3	19	26	9
cer. 0.125% 1 hour . .	45	14	4	23	14	18
cer. 0.125% 2 hours .	49	6	2	26	17	8
control	22	42	20	12	4	62

effective and gave nearly complete control of seedling blight of radish caused by seed-borne *Alternaria spp.*

2. Ceresan-wet at 1.5 % sometimes caused slight symptoms of phytotoxicity, and consequently can better be avoided because of this danger and the higher cost, also. It does not result in a more complete disinfection than is reached by a 1 % solution.
3. With lower concentrations during longer time, a complete disinfection may also be obtained but here the danger of mercury poisoning is very clear.

CHAPTER III

THE LOCALISATION OF THE SEED INFECTION

1. INTRODUCTION

Former workers indicate in their publications, that it is not possible to obtain complete control of Alternaria of radish seed by chemical means. They have drawn the conclusion without exactly proving the fact that at least part of

this infection must be deep-seated, i.e. situated deeper than the superficial layers of the seed coat. Consequently they have turned to the hot-water treatment which is well-known for controlling deep-seated infections in seeds. It was ATKINSON (4) who recently succeeded in isolating the fungus from the inner tissues of radish seed proving that it is deep-seated. In his seed treatment experiments he restricted himself to dust treatments and consequently did not obtain a total control of the disease.

In the present investigation some experiments were carried out to study the relation between the surface infection and the deep-seated infection, and also the relationship between the efficiency of fungicidal treatment and the deep infection.

2. EXPERIMENTS AND RESULTS

In the first experiment of this section, heavily infected radish seed was used. The seed coat was taken off and the naked seeds were placed in blotting paper in a 20 °C incubator of high humidity.

Non-peeled seeds of the same lot were used as a control. One hundred seeds were used for each part of the experiment.

TABLE 8. Percentage of infection in the non-peeled radish seeds compared with the peeled ones

Kind of seed	Seedlings		Non-germ. seeds		Percentage of infection
	healthy	diseased	infected	not-infected	
non-peeled seeds . . .	26	32	16	26	48
peeled seeds	86	2	8	4	10

Consequently about 10 of the 48 % infection is situated deeper than the seed coat, that is somewhat more than 20 %.

The slight attack, resulting in infected seedlings, nearly disappeared by the removal of the seed coat. This means that the infection in the seed coat is responsible for the appearance of slightly attacked seedlings. In the germination test, most of the deep-seated infections result in a severe attack. In this case the symptoms on the non-germinated seeds appear as a greyish-green to brownish-olive mycelium, which envelopes the whole of the seed.

In the next experiment normal seeds were treated with mercuric chloride 0.2 %, for 5 minutes and germinated as such, or after taking off the seed coat. Also non-sterilized seeds with their seed coat were included in the test; 200 seeds were used for each treatment.

TABLE 9. Percentage of infection obtained using non-peeled seed, sterilized non-peeled and sterilized peeled seed

Treatment	Seedlings		Non-germ. seeds		Percentage of infection
	healthy	diseased	infected	not-infected	
non-peeled seeds . . .	46	79	44	31	61.5
sterilized	126	6	17	51	11.5
Ster. peel. seeds	148	0	15	37	7.5

Here we may draw the following interesting conclusions: *Alternaria spp.* can be present in every part of the seed, on the seed surface or superficially in the seed coat layers, deeper within the seed coat cells, and also deep-seated in the inner parts of the seeds.

Corrosive sublimate is suffcient to eliminate the greater part of the infection, which is more or less superficial infection. By removing the seed coat in addition we have got rid of the infection in deeper layers of the seed coat, so that only the really internal infection is left.

In the present experiment only 7.5 in 61.5 % of the infection was situated in the germ itself, which makes 12.1 per 100; so 4 from the seed coat infections are out of reach for the corrosive sublimate, which makes 6.6 per 100; 81.3 % of the total is more or less superficial and can be combated with the fungicide used. From both these experiments it is clear that the seeds with deep-seated infection are among the seeds that fail to germinate.

3. MAKING SECTIONS OF THE DISEASED SEEDS

The results explained in the above are confirmed by making sections of the diseased seeds. According to JOHANSEN (39) it is practically impossible to make sections of entire mature seeds. The trouble is to force the fluids and embedding media to penetrate. The author, however, used Johansen's method with slight alterations and with good success. First the seeds were presoaked in water for four hours. The killing and fixing fluid used was that which is generally known as F.P.A.:

70 % ethyl alcohol	90 cc
propionic acid	5 cc
formaline 40 %	5 cc

In this mixture the seeds remained overnight. Then they were cut at one end in order to enable the fluids to penetrate.

After this they were again put into F.P.A. for a night. After that the seeds were dehydrated by placing them successively in the following volumetric mixtures of water, ethyl alcohol and tertiary butyl alcohol:

distilled water	50	30	15	–	–
ethyl alcohol 95 %	40	50	50	45	–
tertiary butyl alcohol	10	20	35	55	75
ethyl alcohol 100 %	–	–	–	–	25
total alcohol percentage (approximately)	50	70	85	95	100

The seeds were transferred directly from the F.P.A. to the 50 % alcohol mixture; after two hours this was replaced by the 70 % alcohol solution, in which it was allowed to remain overnight. After this came the 85 %, 95 % and 100 % stages for one hour each. The 100 % stage is followed by three changes of pure tertiary butyl alcohol, of which the second is used overnight whereas the first and the third take one hour each. After that the seeds were transferred from the T.B.A. to a mixture of equal parts of paraffin oil and tertiary butyl alcohol, in which they remained for one hour.

A vial three quarter filled with melted paraffin was left until the paraffin had solidified but not completely cooled. Then the seeds were put on top of the

solidified paraffin and just covered with the butyl alcohol paraffin mixture, after which the container was immediately placed in the paraffin oven. On the upper shelf of the oven, paraffin was melted slowly whereas the seeds gradually sank through the paraffin until resting on the bottom on the vial. After one night in the oven the mixture of paraffin and traces of alcohol was poured off and replaced with pure melted paraffin of 45°C melting point. After one night in this a new change of 45°C paraffin was given, in which the seeds had to remain for one hour only. Then this paraffin was replaced by good quality paraffin melting at 52°C for one hour, after which the seeds were ready for embedding. Sectioning was done, according to the method described by Johansen. After mounting the embedded seeds on wooden blocks, it appeared to be of the utmost importance to soak the blocks in water during the night before sectioning. The staining was carried out either with cotton blue and safranin according to LEPIK (9), or with Heidenhain's iron haematoxylin (JOHANSEN, 39), For the results we may refer to the photomicrographs. (Plates I and II).

CHAPTER IV

THE MODE OF ACTION OF CERESAN-WET

1. INTRODUCTION

From the results obtained in testing different fungicides for their relative efficiency it is clear, that only ceresan-wet and germisan-wet gave nearly complete control of the Alternaria infection in radish seed. The dry treatments were not sufficient. In the next experiments only ceresan-wet was used, because both this fungicide and germisan-wet nearly amount to the same in composition and mode of action.

There are two possibilities for explaining the good results with the wet treatment against Alternaria in radish seed. Firstly it may be that the fungicide penetrates into the seeds and kills the fungus. Secondly it is possible that a thin layer of the fungicide on the seed surface prevents the fungus from appearing and sporulating on the outside of the seed. The penetration of a fungicide may be studied by means of a bioassay method. An agar-sheet method for testing the uniformity of fungicide application by different seed treatment machines has been published by MACHACEK (50). A similar method for measuring the actual amount of fungicide on treated seeds has been reported by ARNY & MEAD (3). Using the same principle, experiments were made to compare the quantity of fungicide on seeds treated with ceresan-wet and with ceresan-dry, and also to estimate the remaining fungicide after washing the ceresan-treated seeds in a current of water, or after taking the seed coat away from the treated seeds.

2. MATERIALS AND METHODS

The following procedure was followed in this investigation. Potato-glucose agar in petri dishes was inoculated with *Glomerella cingulata* by means of flooding the agar surface with a heavy spore suspension in water. A sufficient quantity of the suspension was added to cover the whole of the agar surface,

and any excess was poured off by tipping the dishes. The seeds to be tested were placed on the inoculated agar immediately after that procedure. Six seeds from every treatment were used per petri dish, with four replicates. The plates were incubated at 26°C and inspected after 48 hours of incubation. After this time the fungicide from the seed will have more or less diffused into the watery agar, which results in a clear fungus-free zone around the seed. The diameter of this zone depends on the quantity of the fungicide.

3. RESULTS

In this way it was found that the diameter of the inhibition zone around the seed treated with ceresan-wet (1 % solution, 4–8 minutes), is much larger than that obtained with seed treated with ceresan-new (0.3 %) (Photo 8). This means that the quantity of fungicide on the seed after the wet treatment is greater than the quantity of fungicide after the dry treatment, for both products contain nearly the same mercury compound. Untreated seeds, on the other hand, are completely without any inhibition zone whatever.

Another reason why ceresan-wet is so effective against the deep-seated Alternaria infection of radish seed may be, that the mercurial penetrates within the seed coat. To investigate this the second experiment was undertaken. In this experiment the seeds were treated with ceresan-wet and ceresan-new, after which the seed coats were removed, and the naked seeds were placed on the Glomerella-agar surface. The same was done with untreated seeds. No inhibition zone was obtained at all, so we may draw the conclusion that, according to the Glomerella tests, these fungicides do not penetrate within the seeds. Another reason for the different effectiveness of ceresan-wet and ceresan-new against *Alternaria* in radish seed may be, that during the germination test on blotters, the fungicide is more or less washed away from the seeds. This was checked in a third experiment. In this experiment the treated seeds were washed in a current of water for 15 minutes, after which they were plated on Glomerella agar. Photo 9 illustrates the result. Row no. 1 shows the non-treated seed. The second row shows the seeds, treated with ceresan-new and afterwards washed. Here there is no inhibition zone, so that the fungicide dust apparently is easily removed by the washing. The third row demonstrates that after wet treatment and washing, at least a great part of the fungicide is still present on the seeds. Row 4, 5 and 6 give a repetition of the experiment, but with the seed coats taken off before plating the seeds. No zone is obtained anywhere, which is what might be expected: Photo 9.

4. DEMONSTRATING THE PRESENCE OF THE LIVING ALTERNARIA FUNGUS IN THE INTERIOR OF RADISH SEEDS AFTER TREATMENT WITH CERESAN-WET

By means of the Glomerella test, it has been proved that the fungicide does not penetrate into the seed after the treatment. After taking away the seed coat the fungicide could not be demonstrated in the inner tissue. Consequently the fungus must be still alive within the seed. The next experiment will explain this point.

In this blotter experiment seeds treated with 1.5 % and 1.0 % of ceresan-wet were used, firstly with the seed coat, secondly with the seed coat removed immediately after the treatment. Normal untreated seeds of the same sample were used as a control.

TABLE 10. Percentage of *Alternaria spp.* after treatment with ceresan-wet and after peeling the treated seeds

Treatment of seeds	Seedlings		Non-germ. seeds.		% of infection
	healthy	diseased	infected	not infected	
cer.wet 1.5%	72	0	0	28	0
cer.wet 1.0%	73	1	0	26	1
cer.wet 1.5%; peeled .	81	0	8	11	8
cer.wet 1.0%; peeled .	71	0	7	22	7
control	22	32	32	14	64

Thus ceresan in the wet treatment does not kill the fungus immediately after its application: 7 to 8 per 100 seeds, or about 11.7% of the infected seeds are deeply infected and consequently fail to germinate, but still carry the living fungus. This is in accordance with the conclusion of section 2 of this chapter, and confirms the previous result of the Glomerella test.

After reaching this conclusion we still have two possibilities. Firstly the ceresan on the seed coat prevents the fungus from appearing and developing on the seed surface. Secondly the fungicide does not penetrate during or immediately after the treatment, but does so during the germination when the seed has to absorb water from its surroundings. In the next experiment we will go into this point.

Seed was treated with ceresan-wet in different ways and germinated in the ordinary blotter test. A certain number of the treated seeds failed to germinate, and the question now is whether or not these seeds still contain the living fungus at the end of the germination test. In order to decide this, the seed coat of the non-germinated seeds after every treatment was taken off, and then these peeled seeds were again put on a moist blotter for 5 days at 20°C.

TABLE 11. Efficiency of ceresan-wet against the deep-seated infection during germination of the treated seeds

Treatment	Seedlings		Non-germ. seeds		% of infection
	healthy	diseased	infected	not infected	
1% cer.wet 1 min. . .	74	4	0	22	4
1% cer.wet 2 min. . .	78	2	0	20	3
1% cer.wet 4 min. . .	80	0	0	20	3
1% cer.wet 8 min. . .	78	0	0	22	2
2% cer.wet 1 min. . .	77	1	0	22	3
control	21	46	17	16	63

The 22, 20, 20, 22 and 22 non-germinated seeds, respectively, were put on a moist blotter for the second period after removal of their seed coats, and in this way showed 4, 3, 3, 2 and 3 of their number with Alternaria growth, respectively.

Consequently of these 106 treated and in the subsequent test non-germinated seeds, 15 still contained the pathogen in a living state at the end of the germination test. Comparing this result with that of Table 10, in which 7.5% of all the seeds were deeply infected, we can calculate that 37* of these 106 seeds

* $\frac{7.5 \times 500}{100} = 37$

have carried the deep-seated infection, so that at least part of this deep-seated infection has been killed during the germination test. The lesser half survived the treatment during the subsequent germination. During the germination test after the wet treatment, part of the deep-seated infections are killed, and part of them are not killed but do not have the possibility of developing on the outside of the treated seed. Both the possibilities mentioned above are playing a role.

An experiment was also conducted with storage of the seed in a closed bottle for different times between treatment and germination test, with the purpose of studying the influence of the storing on the deep-seated infection. So the seeds were treated, stored, peeled and then immediately germinated. The results of this experiment are given in detail in Table 12.

TABLE 12. Percentage of infection in the seeds, peeled after the period of storage and just before putting them in the germination test

Treatment cer.wet 1 % 8 min.	Normal seedlings		Abnormal seedlings	Non-germ. seeds		% of infection
	healthy	diseased		infected	not infected	
no storage	113	0	48	8	31	4
1 day stored	115	0	42	3	40	1.5
2 days stored	121	1	41	2	35	1.5
4 days stored	113	0	49	1	37	0.5
8 days stored	107	0	49	1	43	0.5
15 days stored	82	0	65	0	53	0
32 days stored	76	0	61	0	63	0

After every storage period 200 seeds were tested. A column of abnormal seedlings has to be included in this table, as during the storage period a certain amount of mercury poisoning was caused. These seedlings did not show any Alternaria infection, so that a distinction between healthy and diseased had not to be made here.

From this experiment we may conclude, that during the storing of the treated seed the effect of the fungicide is accumulated. The deep-seated infection is 4 % at the start and decreasing to zero in about two weeks. At the same time the germinating capacity of the seeds is also affected by the phytotoxic activity of the fungicide during storage.

It must be pointed out, that in this experiment another seed sample was used than in the preceding experiments, so that the figures of the three different experiments in this section cannot be compared directly.

CHAPTER V

THE USE OF ANTIBIOTICS FOR CONTROLLING THE DISEASE

1. INTRODUCTION

Antibiotics are of great theoretical interest and potential practical importance in plant pathology. During their relatively brief history they have been tried for several purposes, including the treatment of seeds. The first report of the use of an antibiotic as seed treatment material is that of BRIAN & HEMMING,

who in 1945 demonstrated that gliotoxin has fungitoxic activity in tests with small grain diseases (7). Since then a number of antibiotics have been used for this purpose and many reports have been published that deal with the treatment of seeds or tubers with such substance in greenhouse or field experiments (1, 2, 35, 45, 30, 36, 76, 78, 80). These papers record a number of instances of effective disease control with antibiotics, but as yet these materials have not come into general use for treating seeds.

The aims of treatment are twofold: to prevent disease development by pathogens that may be carried on or in the seed, and to protect seeds and seedlings from attack by soil-borne plant pathogens.

WALLEN & SKOLKO (79) reported, that antibiotics gave a good control of the deep-seated fungus *Ascochyta pisi* in pea seed. HENRY *et al.* (30) soaked oats and barley seed for four hours in a cycloheximide solution and in this way obtained field control of the covered smuts of these cereals. Tests with wheat bunt were also successful, although the seed was injured. In a later paper, workers at the same station reported having obtained control of bunt with 20 cycloheximide by means of a 1 minute soaking period and even by dust treatments (31).

Streptomycin has been used for seed-treatment purposes, particularly for the control of bacterial diseases. ARK (2) reported, that the soaking of cucumber seeds, that were infected with the angular leaf spot bacterium, in streptomycin solutions was an effective control practice. In a greenhouse test, bean blight, likewise caused by a bacterium within the seed, also appeared to have been controlled by a streptomycin soaking treatment (34, 72). This antibiotic has in addition been claimed to have reduced the loose smut disease of barley, which is caused by a fungus that is carried within the embryo of the seed (60).

A number of other antibiotics have been used for seed treatment. In laboratory experiments antibiotic XG has been reported to control the Ascochyta fungus in pea seeds (79). Helixin B has been found to be effective for the control of certain Helminthosporium diseases of oats and barley, and likewise for three covered smuts of small grains (45). Two related antibiotics from the Wisconsin laboratories, antimycin A-35 and antimycin A-102, have been shown to provide a degree of control for certain oat diseases (44, 49).

Presumably pure or partly pure antibiotics were used by KRASILNIKOV (43) and by MIRZABELSYAN (58), who reported the successful treatment of cotton seed for the control of the angular leaf spot disease. Culture fluids of microorganisms have been reported to be effective for disease control in seed treatment studies with *Sclerotinia libertiana* (14), *Pseudomonas tabaci* (14) and *Tilletia tritici* (13). DEKKER (16) reported that soaking the pea seed in a solution of 100 p.p.m. rimocidin for 18 hours gave nearly complete control against *Ascochyta pisi*. By soaking heavily infected seed in rimocidin 100 p.p.m. for 24 hours the number of seed-borne diseased plants in glasshouse experiments could be reduced from 40 to 1.5 % without deleterious effect on germination (59, 17).

In combating soil-borne diseases, the emphasis has been laid by workers on treatment of the soil, from which they seem to expect better possibilities than from treatment of the seed itself. Tests with antibiotics for soil treatment have been reported by GREGORY *et al.* (26), who found that the damping-off of alfalfa seedlings was prevented by adding cycloheximide solutions to the soil. However, the antibiotic also inhibited plant growth. JOHANSEN (40) reported that antibiotic PF was not effective for the control of *Rhizoctonia solani*.

2. MATERIALS AND METHODS

The antibiotics listed below were used as dry treatment at the rate of 0.3 %.

Material	Producing organism	Firm
terramycin	*Streptomyces rimosus*	Chas Pfizer & Co. Inc., New York
rimocidin	*Streptomyces rimosus*	
agrimycin	Streptomycin 15 % + terramycin 1.5 %	
actidione	*Streptomyces griseus*	Pfizer & Co, Inc. N.Y. Up John, Kalamazoo
aureomycin	*Streptomyces aureofaciens*	
penicillin	*Penicillium notatum* and other species of *Penicillium* and *Aspergillus*	many firms
vengicide	*Streptomyces spec.*	Ned. Gist- en Spiritus-fabr. Delft. The Netherl.

3. EXPERIMENTS AND RESULTS

In the first experiments of this kind the antibiotics agrimycin, aureomycin, penicillin, terramycin and vengicide were included, but all of these completely failed to control the Alternaria infection of radish seed, even the superficial infection. This will be clear from the following table, in which two experiments are combined, with 400 seeds for the first four antibiotics and 200 for the fifth.

TABLE 13. Efficiency of some antibiotics against *Alternaria spp.* in radish seed

Treatment	Seedlings		Non-germinated seeds	% of infection	% of infection of the control
	healthy	diseased			
agrimycin	247	136	17	34	32
aureomycin	250	135	15	33	32
penicillin	268	126	6	31	32
terramycin	265	129	6	32	32
vengicide	64	102	34	51	46

This negative result is not so surprising, as several of the products used are exclusively bactericidal.

In a following experiment actidione was used in solution during different times, with 100 seeds per treatment.

TABLE 14. Efficiency of actidione 100 p.p.m. on *Alternaria spp.*

Treatment with actidione 100 ppm.	Seedlings		Non-germ. seeds		Abnormal seedl.	% of infection	% of germination
	healthy	diseased	infected	not infected			
1 hour	23	18	4	30	25	22	41
2 hours	25	14	2	29	30	16	39
4 hours	8	4	5	57	26	9	12
8 hours	17	0	0	61	22	0	17
16 hours	6	0	0	66	28	0	6
control	21	36	25	11	7	61	57

From the above table it is clear, that actidione is effective for controlling the Alternaria infection, but in the concentration used, only when applied during 8 hours at least. On the other hand it is phytotoxic to the radish seed, so it cannot be used in practice as a disinfectant against *Alternaria spp.* in radish seed.

The following experiments were concerned with rimocidin.

Table 15 is composed from two experiments with the same seed sample, in one of which 200 seeds per treatment were used, whereas in the other 100 seeds per treatment were used. Ceresan-wet was included for comparison.

TABLE 15. Effect of using different concentrations of rimocidin for different times to control *Alternaria spp.* in radish seed

Treatment with rimocidin	Seedlings		Non-germ. seeds		Abnormal seedl.	% of infection
	healthy	diseased	infected	not infected		
200 p.p.m. 1 hr	60	13	5	13	9	18
200 „ 2 hrs	57	8	6	13	16	14
200 „ 4 hrs	59	6	5	20	10	11
200 „ 8 hrs	66	6	4	13	11	10
200 „ 16 hrs	71	0	0	20	9	0
200 „ 20 hrs	112	0	0	41	32	0
200 „ 24 hrs	126	0	0	38	36	0
400 „ 1 hr	99	16	16	31	38	16
400 „ 2 hrs	114	10	1	28	47	5.5
400 „ 4 hrs	129	5	0	38	28	2.5
400 „ 8 hrs	61	0	0	18	21	0
400 „ 16 hrs	68	0	0	10	22	0
800 „ 1 hr	120	9	4	30	37	6.5
800 „ 2 hrs	128	1	0	34	37	0.5
800 „ 4 hrs	125	0	0	36	39	0
800 „ 8 hrs	126	0	0	35	39	0
800 „ 16 hrs	121	0	0	40	39	0
cer. 1% 5 min.	118	2	0	41	39	1
control	21	36	25	11	7	61

In the following table these results are summarized:

TABLE 16.

Concentration of rimocidin	Duration of treatment (hours) and percentage of infection						
	1	2	4	8	16	20	24
200 p.p.m. .	18	14	11	10	0	0	0
400 „ . .	16	5.5	2.5	0	0	0	0
800 „ . .	6.5	0.5	0	0	0	0	0

Thus this antibiotic offers good possibilities for controlling the infection. In the case of a lower concentration the time of soaking has to be longer for complete control, but even the highest concentration used during the longest time was not phytotoxic.

4. COMPARISON BETWEEN THE MODE OF ACTION OF CERESAN-WET AND RIMOCIDIN

From previous experiments it appeared, that ceresan-wet does not penetrate into the seed and kill the deep-seated fungus immediately after the treatment. During the subsequent germination, however, it does penetrate to some extent and consequently partly kills the deep-seated infection. In the present investigation and after the success obtained with the antibiotic rimocidin, it will be necessary to compare the mode of action of this substance with that of ceresan-wet.

Firstly we will have to see whether rimocidin penetrates within the seeds and kills the deep-seated infection immediately after treatment or not.

TABLE 17. Comparison between the efficiency of ceresan-wet and rimocidin on deep-seated *Alternaria spp.* in radish seed

Treatment	Seedlings		Non-germ. seeds		Abn. ormal seedl.	% of infect tion
	healthy	diseased	infected	not infected		
non-treated seed . . .	42	72	50	22	14	61
the same, peeled . . .	123	4	20	19	34	12
1 % cer. 6 min.	118	2	0	41	39	1
the same, peeled . . .	149	1	9	12	29	5
rimocid. 200 p.p.m. 18 h.	112	0	0	41	32	0
the same, peeled . . .	137	0	9	18	36	4.5

From this table it appears, that ceresan-wet and rimocidin are equal as to the control of the deep-seated fungus. Thus after the ceresan-treatment about 5 % deep-seated infection remains hidden beneath the seed coat, out of reach for the fungicide and making its appearance only after the removal of the seed coat. After the treatment with rimocidin the comparable figure is 4.5 %, which is practically the same.

So rimocidin 200 p.p.m. during 18 hours is at least as good as ceresan-wet 1 % for 6 minutes for combating the infection, but both treatments do not reach the deep-seated Alternaria infection in radish seed.

The next point is whether rimocidin is capable of reaching the deep-seated fungus during the germination test. In the comparable experiment with ceresan-wet (Table 11) it was shown that this fungicide partly suppressed the deep-seated infection during the germination and partly failed to do so. For the present experiment the heavily infected seed was treated with either ceresan-

TABLE 18. Comparison between the mode of action of ceresan-wet and rimocidin on deep-seated *Alternaria spp.* during the incubation period

Treatment	Seedlings		Abnormal seed-lings	Non-germ. Altern. free	The same with Altern. after peeling
	healthy	diseased			
cer. wet 1 % 6 min. . .	119	2	41	38	3
rimocidin 200 p.p.m. 24 h.	126	0	36	38	0
rimocidin 200 „ 20 h.	127	0	32	41	0
rimocidin 400 „ 16 h.	136	0	44	20	0

wet or with rimocidin, and afterwards incubated for seven days as usual. After this germination period the seed-coat of the seeds that had not germinated, was removed and these naked seeds were again incubated for 7 days. Table 18 shows the results of both incubations.

From the above experiment it is clear that the treatment with ceresan-wet killed part of the deep-seated Alternaria infections during the first period of incubation. After removing the seed coat and incubating for a seven further days, 3 of these seeds again showed Alternaria development. In the case of treatment with rimocidin, no development of *Alternaria spp.* was observed after the removal of the seed coat and the second period of incubation. This indicates that rimocidin is capable of killing all of the deep-seated Alternaria infection in radish seed during germination after the treatment, whereas ceresan-wet is only partly capable of doing so.

The third experiment has to be compared with that of Table 12. In the experiment of Table 12 the effect of the treatment with ceresan-wet was increased by storage of the treated seed in stoppered bottles, because the possibility of increasing the dosages of the mercurial did not exist in relation to its toxic effect.

For rimocidin, storing the treated seed does not offer so many possibilities, as the antibiotics more quickly looses its activity. However, rimocidin can be administered in higher concentrations and during a longer time of soaking. This gave the following figures (Table 19) in an experiment, in which the seed coats from all the seeds were removed immediately after the treatment with rimocidin.

TABLE 19. Efficiency of rimocidin 400 p.p.m. for different times on *Alternaria spp.* in radish seed

Treatment with rimocidin	Seedlings		Non-germ. seeds		Abn. seed-lings	% of infec-tion
	healthy	diseased	infected	not infected		
400 p.p.m. 40 h. . . .	112	0	0	26	62	0
400 „ 30 h. . . .	116	0	0	19	65	0
400 „ 20 h. . . .	121	0	1	22	56	0.5
400 „ 10 h. . . .	117	0	2	20	61	1
400 „ 5 h. . . .	118	0	8	17	57	4
400 „ 2½ h. . . .	114	0	7	21	58	3.5

This demonstrates that it is very difficult to remove the last trace of the infection. Soaking the seed in 400 p.p.m. for 30 to 40 hours is, however, sufficient to kill all of it immediately. The same result is obtained by 15 days storage after treatment with 1 % ceresan-wet for 8 minutes. The 10 to 20 hours level with rimocidin 400 p.p.m. more or less agrees with 1 to 8 days storage after treatment with 1 % ceresan-wet for 8 min.; further 2½ to 5 hours treatment with the antibiotic agrees with the ceresan-wet treatment without storage. This rimocidin treatment, however, is not phytotoxic to the seed, whereas the ceresan-wet treatment becomes phytotoxic during the storage period.

5. COMPARISON BETWEEN CERESAN-WET AND RIMOCIDIN IN GREENHOUSE EXPERIMENTS

In a first test, treatments with ceresan-wet 1 % 8 minutes and rimocidin 200 p.p.m. 18 hours were compared in sterile soil. A loamy sand was used, semi-sterilized by steaming, in flat pots with a very thin covering layer. The greenhouse temperature was about 15° to 20°C. The pots were not covered, so that it was necessary to spray them every day with water. The test was finished after 10 days. The following figures were obtained:

TABLE 20. Percentage of germination of radish seed treated with ceresan-wet and rimocidin

Treatment	Normal seedlings	Abnormal seedlings	Dead seeds	% of germination
ceresan 1% 8 min.	120	15	65	60
rimocidin 200 p.p.m. 18 h.	128	17	55	64
control	103	10	87	51.5

Thus in this experiment, with circumstances very favourable for germination (good temperature, sufficient moisture) there is indeed a difference between the treatments and the control, but this difference is not great. The difference between ceresan-wet and rimocidin is very small.

In a following experiment the soil was kept constantly wet by placing the pots in a tray with water, and covering the whole with glass. Now the differences between the treatments and the control were far greater:

TABLE 21. Percentage of germination of seed treated with ceresan and rimocidin under circumstances favourable to *Alternaria spp.*

Treatment	Normal seedlings	Abnormal seedlings	Daed seeds	% of germination
ceresan 1 % 8 min.	120	26	54	60
rimocidin 400 p.p.m. 18 h.	118	25	57	59
control	64	0	136	32

Under these circumstances the Alternaria fungus apparently has better possibilities for development.

Previous investigators as a rule have not found appreciable differences in emergence from treated and non-treated radish seed. This probably will have been due to the use of drier soil in their greenhouse and field experiments.

PART II

RELATIVE VALUE AND MODE OF ACTION OF THIRAM AND MERCURIALS USED AS SEED PROTECTANTS AGAINST SOIL FUNGI

CHAPTER VI

INTRODUCTION, AND MATERIALS AND METHODS

1. INTRODUCTION

Chemical seed treatment was originally introduced with the purpose of killing fungus spores on the seed surface, but has since been used for a much wider range of purposes. Promising modern fungicides of different chemical composition have been tested for seed treatment, and a few of these have proved particularly suitable as protective dressings, e.g. for preventing damping-off of seedlings as a consequence of the activity of soil fungi.

Thiram (TMTD) probably has the widest range of uses and is the most versatile. There is a current interest in fungicides of the dithiocarbamate group because of their proved value for combating sugar beet rootrot (32, 33, 55), damping-off of certain vegetable species, and other purposes (19, 54, 73).

Organic mercurials are decidedly superior to materials of the thiram group for combating seed-borne diseases like cereal smuts (11), and according to the first part of this thesis mercurials are also preferable for combating *Alternaria spp.* in radish seed. CRONCHEY also compared the value of copper compounds, mercurials and thiram against seed- and soil-borne infections of beet seedlings. In his experiments thiram affords superior protection against post-emergence damping-off due to various causes.

The aim of the present study was to obtain more details concerning the chemical protection of seeds by comparing the mode of action of different types of fungicides. The activity of different fungicides, used either for seed or for soil treatment, against soil-borne micro-organisms was studied, as well as the characteristics of their action and persistence on the seed and in the soil.

For this work it was necessary to use a seed species that is sensitive to soil micro-organisms, and to have a simple and rapid method for testing the protective action of the materials. The choice fell on corn, for which crop there is a rapid and reliable laboratory method of testing available in the American cold-test (38, 81). This method proved very useful for illustrating the relative value and action of mercurials and thiram, as protectants against soil-inhabiting damping-off fungi. In this crop it is easy to find seed lots, for which seed-borne diseases can safely be neglected, so that only plant pathogens from the soil are playing a role, and consequently only protection by the fungicide is necessary. A short explanation concerning the cold-test may be useful. Cold-testing of corn has been described as "the germination of corn under adverse conditions, to determine the resistance of inbred and hybrid seed lots to seedling blight". Such a testing method has particular value for plant breeders as well as for seed merchants, who wish either to test the cold resistance of new strains, or to know the sowing value of individual seed lots. Several of the companies that are engaged in producing and marketing hybrid corn varieties, have

especially equipped laboratories, which are devoted entirely to cold testing and in which thousands of tests are made every year. The method is also used by seed testing stations and experimental stations. The principle was indicated by DICKSON, who laid the foundation for modern cold testing. His experiments were performed to discover the temperature range at which maize was most severely attacked by *Gibberella zeae* (Schw.) Petch (18).

WERNHAM (81) and also ISELY (38) have reviewed cold testing methods and some of the results obtained by their use. The former reported that seventeen different fungi have been accused of causing seedling blight of corn under adverse conditions. Eight of these are species of *Pythium*, five are *Helminthosporium* spp., and also *Gibberella zeae* (Schw.) Petch, *Diplodia zeae* (Schw.) Lev., *Penicillium oxalicum* and *Rhizoctonia solani* Küehn may be active in this way.

The present-day methods of cold-testing are all based on the same principle, i.e. exposing maize kernels in unsterilized field soil to low temperature, which inhibits seed germination but does not prevent the activity of the soil fungi. Mostly a temperature of 10°C is used for 5 to 7 days, and afterwards the boxes are moved to a warm germination chamber or a greenhouse, held at a temperature close to 30°C, in which germination is allowed to proceed normally. Determination of the percentage of germination is made after another 3 to 5 days. The percentage of the seed that fails to produce normal seedlings, is a measure for the liability of the seed sample to pre-emergence blight.

Normally the soil is taken from fields on which maize has been grown for one of more years, in order to make sure that the necessary soil-micro-organisms occur in sufficient number. This comes down to a conditioned laboratory imitation of a normal field sowing. So far the results obtained with the cold-test suggest that the use of natural field soil with its complicated soil flora is preferable to the use of some artificial medium.

RICE suggested, that a cold-test should not only be of value for comparing corn seed lots but also for evaluating fungicides and dressing methods (65).

In the present investigation the cold test technique was used for determining the thiram and the mercurial content of treated seed and treated soil.

For the behaviour of thiram in treated soil we may refer to the study of RICHARDSON (66) who used the *Glomerella* agar technique for this purpose.

2. MATERIALS AND METHODS

For the investigations only a single seed lot was used, which was procured with the help of the Dutch Seed Testing Station. In the routine investigations of commercial samples by this Institute, this seed lot had shown to have a germinating capacity (in pure sand) of nearly a hundred percent, in a cold-test untreated of about 20 %, and in the same after thiram treatment of about 90 %. So this seed lot had proved to be very weak and reacting very favourably to fungicide treatment, which made it extremely well adapted to fungicide investigation.

Of this lot several quantities were obtained in the course of time. It is not sure that these quantities were precisely alike but all of them proved to have comparable properties.

For the cold-test a type of loamy sand was used from a corn field. The water holding capacity of this soil was about 30 % and it was normally used with a water content of 8 to 9 %.

The testing was performed in shallow zinc trays of about 10 × 25 cm, with a perforated bottom, as used for the blotter tests with radish seed. The trays were provided with a thin layer of soil (with a blotting paper underneath); this was provided with 75 hollows per tray, by means of a board having 75 nails with button heads. The individual seeds were placed in these holes and were then covered by another thin layer of soil and another wet filter paper. The total amount of soil per tray was about half a kilogram, and the thickness of the two layers together nearly 2 cm.

The trays were placed in a moist incubator at 10°C. In certain experiments they were enveloped in poly-ethylene plastic. After 7 days in this incubator they were transported to a dark germinator at 28°C, and after a further three days the seedlings were judged and counted. Except when plastic covering was used, the trays were sprayed every day in order to keep the moisture content at the original level. In finishing the test the emerged seedlings were judged especially for their root system, and those with insuffcient root formation were discarded and not included in the emergence-percentage. With the seed lot used the number of abnormal seedlings per tray, however was very low.

With this method, which may seem rather crude, the results were very regular and reliable.

In certain experiments the cold-test results were replenished with information obtained by the Glomerella agar test, for a detailed description of which we may refer to Chapter IV section 2.

CHAPTER VII

RELATIVE EFFICIENCY OF DIFFERENT FUNGICIDES AGAINST DAMPING-OFF OF CORN

To begin with, a series of fungicides were tried with the cold-test method using the corn seed sample mentioned before. For the fungicides used we may refer to Table 1. The resulting emergence percentages are given in Table 22.

TABLE 22. Relative effectiveness of different fungicides in the cold-test for corn

Fungicide and dosage	Number of normal seedlings	Number of abn. seedlings & dead seeds	Percent. of germination
0.3% arasan SF-X dry	121	29	80.6
0.3% aapirol-80 dry	118	32	78.6
0.3% aatiram dry	116	34	77.3
0.3% lirothiram dry	112	38	74.6
2.0% tripomol-wet	107	43	71.3
0.3% aagrano dry	106	44	70.6
0.3% panogen liquid	105	45	70.0
0.3% ceresan-new dry	103	47	68.6
1.0% ceresan-wet (8 min.)	76	74	50.6
2.0% ceresan-wet (8 min.)	abnormals owing to phytotoxicity		
non-treated in field soil (control)	58	92	38,6
non-treated seed in glass sand	121	29	80.6

In this and the following experiment, undertaken in the beginning of the

summer, a good supply of soil had not yet arrived. The soil used had an insufficient microbiological activity, in comparison with the soil type, that was used afterwards.

These results show that the tetramethylthiuram compounds (i.e. the first five products of the table) were most effective as protectants. The organic mercury compounds came second in reducing seedling blight of corn caused by the micro-organisms of the soil. Arasan SF-X appeared to be the most effective product. The result with glass sand, which is virtually sterile, shows that the reduction in emergence is the consequence of the properties of the soil, in all probability of the activity of the soil fungi. It is indicated that low temperature in itself was not injurious, but served merely to predispose unprotected slow-germinating kernels to attack by soil fungi.

In the following experiment, different high dosages of two chosen fungicides are compared, i.e. of the thiram compound arasan SF-X and the mercurial ceresan-new.

TABLE 23. Relative efficiency of treating corn seed with high dosages of ceresan-new and arasan SF-X against damping-off

Rate of application	Arasan SF-X			Ceresan-new		
	normal seedlings	dead & abnormal	percent. of germin.	normal seedlings	dead & abnormal	percent. of germin.
0.8%	114	36	76.0	67	83	44.6
0.4%	106	44	70.6	74	76	49.3
0.2%	105	45	70.0	97	53	64.6
0.1%	105	45	70.0	81	69	54.0
control	34	116	22.6	34	116	22.6

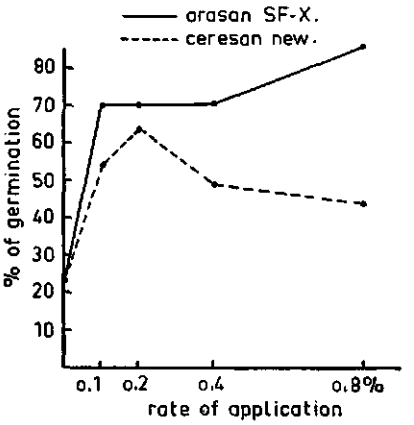
Next follows an experiment, which forms a continuation of the preceding one, but which has been undertaken with other seed, and a new batch of microbiologically more active soil.

Consequently the figures are not directly comparable, but for each experiment they have to be compared with their own control (in fact this experiment has been undertaken very late).

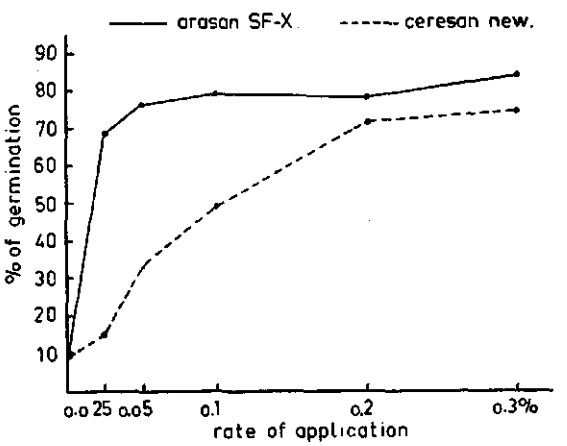
TABEL 24. Relative efficiency of treating corn seed with low dosages of ceresan-new and arasan SF-X against damping-off

Rate of application	Arasan SF-X			Ceresan-new		
	normal seedlings	dead & abnormal	percent. of germin.	normal seedlings	dead & abnormal	percent. of germin.
0.3%	378	72	84.0	336	114	74.6
0.2%	354	96	78.6	323	127	71.7
0.1%	358	92	79.5	221	229	49.1
0.05%	344	106	76.4	152	298	33.7
0.025%	308	142	68.4	68	382	15.1
control	42	408	9.3	42	408	9.3

From these tables and graphs it can be observed that arasan gives better results than ceresan-new in protecting corn seed against soil fungi in all the dosages used.



GRAPH 3. Relative efficiency of treating corn seed with high dosages of ceresan-new and arasan SF-X against damping-off.



GRAPH 4. Relative efficiency of treating corn seed with low dosages of ceresan-new and arasan SF-X against damping-off.

When both arasan and ceresan-new were used in the normal dosages of 0.2 and 0.3 % their difference in efficiency was not so great as when applied in higher or in lower dosages. In high dosages only ceresan-new proved to be phytotoxic, having a decreasing influence on germination. In the lower dosages ceresan-new shows a decreasing effect from an emergence of 74.6 % at the dosage of 0.3 %, to an emergence of 15.1 % at the dosage of 0.025 %. In the case of arasan SF-X a certain decrease in emergence is also visible over the same range of dosages, but this decrease is very slow and gradual.

CHAPTER VIII

COMPARISON OF PERSISTENCE OF ARASAN SF-X AND CERESAN-NEW ON CORN SEED

1. PERSISTENCE DURING STORAGE

In this investigation corn seed was treated with arasan SF-X and ceresan-new in a dosage of 0.3 %, after which the seed was stored in large open petri dishes at room temperature. The amount of fungicide on the seed had to be comparatively determined after different periods of storage. For this purpose both the Glomerella agar test and the cold-test were used.

TABLE 25. Persistence of fungicides on corn seed (Glomerella agar test) – numbers indicate diameter of inhibition zone in mm)

Fungicide and dosage	Time of storage in days							
	0	1	2	4	8	15	31	60
arasan 0.3 %	26.5	26.2	29.8	27.2	26.9	29.0	29.1	20.0
ceresan 0.3 % . . .	12.4	11.2	11.4	11.0	12.6	12.8	4.8	0.0
non treated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The results indicate the shortest distance from the seed till the border of the hyaline zone, in which one has to take into consideration that the zone is often somewhat irregular.

TABLE 26. Persistence of fungicides on corn seed (cold-test) numbers indicate percentage of germination

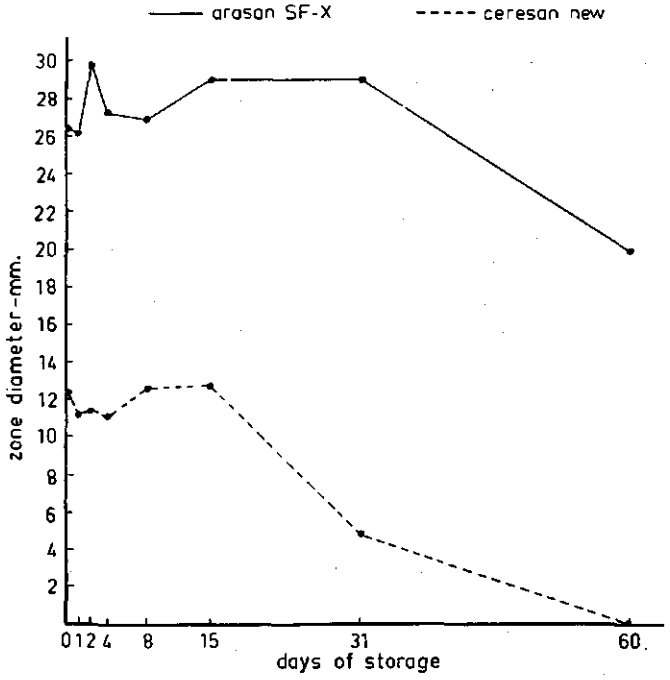
Fungicide and dosage	Time of storage in days							
	0	1	2	4	8	15	31	60
arasan 0.3 %	93.3	93.3	88.0	96.6	90.6	96.0	94.6	69.3
ceresan 0.3 % . . .	78.6	94.6	88.0	96.0	93.3	88.0	18.0	4.0
non-treated	12.6	10.0	8.6	5.6	9.3	8.6	11.6	5.6

From these tables and graphs it is clear, that when the action of ceresan-new is being determined with the Glomerella agar test, the inhibition zone begins to decrease in size after more than 15 days, decreases from an average of 12 mm to 4.8 mm after 31 days, and reaches zero after 60 days of storing. In the case of arasan SF-X the inhibition zone did not decrease until after 60 days of storage, when it had dropped from an average of 27.8 mm to 20.0 mm diameter.

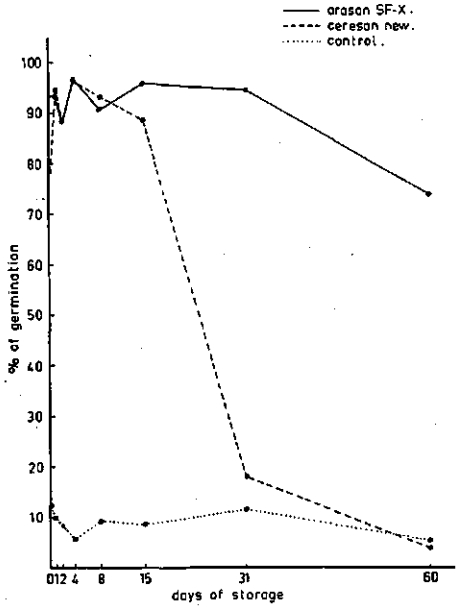
These results were nicely confirmed by the cold-test. Here the percentage of germination in the case of ceresan-new decreased from an average of 89.7 to 18.0 % and 4.0 % after 31 and 60 days, respectively. In the case of arasan SF-X it did not decrease until after 60 days' storage, when it had fallen from an average of 93.4 to 69.3 %.

Apparently the thiram compound persists longer on the seed surface than does the mercury compound, when the treated seed is stored in open air.

In Table 26 ceresan-new starts with a lower emergence figure in the cold-test (78.6 %), whereas this is not the case with arasan SF-X. Perhaps this is caused by



GRAPH 5. Persistence of fungicides on corn seed (Glomerella agar test).



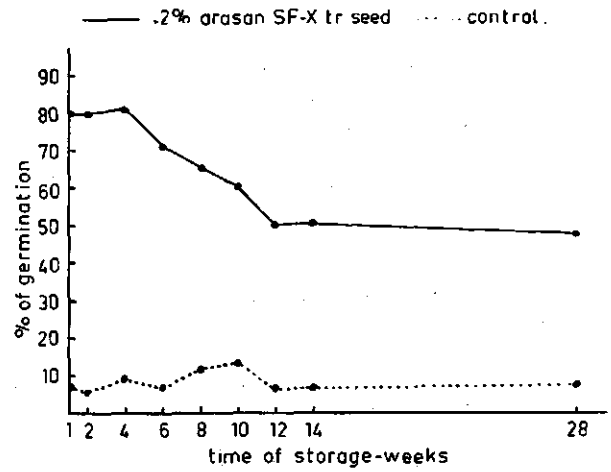
GRAPH 6. Persistence of fungicides on corn seed (cold-test).

the mercurial needing some time for adsorption into the kernel surface, whereas this is not the case with thiram which apparently begins its action immediately.

In the following experiment only the cold-test was used, the dosage was only 0.2 % of arasan SF-X, and the total duration of the experiment was increased to 14 weeks of storing. Table 27 gives the results.

TABLE 27. Persistence of arasan SF-X on corn seed (cold-test) dosage of 0.2 %

Treatment of seed	Time of storage-weeks								
	1	2	4	6	8	10	12	14	28
arasan SF-X	80.0	80.0	81.3	71.3	66.0	60.6	50.6	51.3	48.0
non-treated seed (contr.)	7.3	5.6	9.3	7.0	12.3	14.6	7.6	7.3	8.6



GRAPH 7. Persistence of arasan SF-X on corn seed (cold-test).

In this long storage experiment the efficiency of arasan SF-X on the stored treated seed started to decrease already in the sixth week of storage, and continued gradually until 12 weeks. After 12 weeks the efficiency of arasan SF-X reached a certain level and stayed until the end of the experiment without showing any further decrease (this was indicated by the diminishing emergence of the cold-tested seed from an average of about 80 % to 71, 66, 60.6, 50.6, 51.3 and 48.0 % after 6, 8, 10, 12, 14 and 28 weeks of storage respectively).

2. PERSISTENCE OF FUNGICIDES ON CORN SEED AFTER PLANTING

For studying this subject in a preliminary experiment the seed was treated with 0.3 % of ceresan-new and arasan SF-X respectively, and then planted as usual in natural soil in shallow trays that were covered with moist filter paper and stored in the 10 °C incubator. Enough water was added every day to keep the moisture content of the soil approximately at the original level. The loss of fungicide from the seed was estimated by taking individual seeds with tweezers out of the soil after different periods of time, and transporting them to Glomerella agar dishes.

The next table shows the results of this experiment.

TABLE 28. Persistence of fungicides on corn seed after planting (Glomerella test) – numbers indicate diameter of inhibition zone in mm

Fungicide and dosage	Number of days in soil					
	before planting	1	2	4	8	20
arasan SF-X 0.3 % . . .	61.0	59.2	56.9	57.3	57.2	59.0
ceresan-new 0.3 % . . .	40.8	15.0	10.8	0.0	0.0	0.0
untreated	0.0	0.0	0.0	0.0	0.0	0.0

Here the inhibition zone was measured in two directions, and the average of the two diameters was taken as the zone size. Every figure is the average of the zone size in 10 petri dishes. With ceresan the zone was already eccentric after 1 and 2 days in the soil, so that the Glomerella growth reached one end of the seed.

These results throw light on the mode of action and behaviour of ceresan-new and arasan SF-X, after planting the treated seed in soil. Thiram persists on the seed for a much longer time than the mercury compound. The inhibition zone in the Glomerella test did not decrease until 20 days after planting in the case of arasan SF-X, whereas in the case of ceresan-new it already diminished very much after one and two days in soil (from 40.8 mm to 15.0 and 10.8 mm) and in four days the fungicide had totally disappeared.

The following experiment was tried to determine whether the fungicide in disappearing from the seed had disappeared altogether, or had migrated to the adjoining soil.

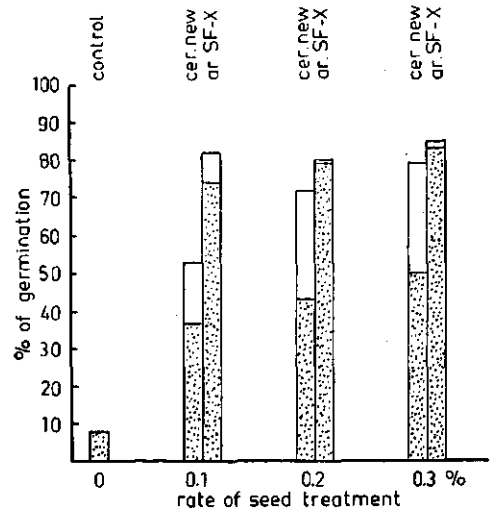
Corn seed was treated with ceresan-new and with arasan SF-X in dosages of 0.1, 0.2 and 0.3 %. Three days after treatment the seeds were planted as usual, i.e. in natural soil in the shallow cold-test trays, and incubated for 24 hours in the 10°C incubator. After this period of 24 hours the seeds were very carefully taken out of their holes and immediately replaced by fresh untreated seeds, whereas the "old" seeds were transferred to fresh trays with naturally-infested soil.

In this way it would be possible to detect the fungicide that might have moved from the old seeds into the surrounding soil, and to determine the amount of fungicide still adhering to the seeds by trying these in a new cold-test.

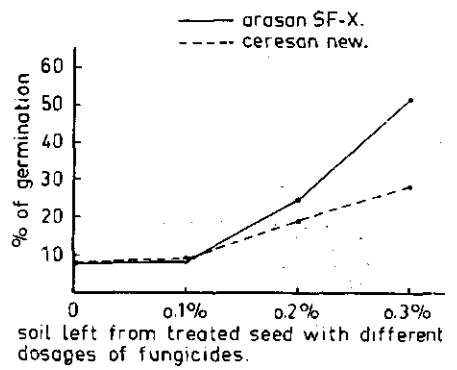
TABLE 29. Behaviour of fungicides after planting the seeds in soil – numbers indicate percentage of germination. Untreated control 8.6 % of germination

Description experiment	Fungicide dosage		
	0.1 %	0.2 %	0.3 %
arasan-treated seed, not transferred . .	82.6	80.0	85.3
arasan-treated seed, transf. to fresh soil .	74.3	79.6	83.3
fresh seed replacing arasan-tr. seed . . .	8.9	25.0	51.6
ceresan-treated seed, not transferred . .	53.3	72.6	79.3
ceresan-treated seed, transf. to fresh soil .	36.9	43.0	50.0
fresh seed replacing ceresan-tr. seed . .	9.0	19.6	28.3

Half of the seed was left in its place as a control, that is for comparing the efficiency of the normal treatment with the efficiency of the fungicide that had migrated to the surrounding soil, and with the efficiency of the fungicide that had stuck to the seed.



GRAPH 8. Persistence of fungicides on the seed after planting. Total height of the columns indicates the cold-test emergence of non-transferred seed; dotted columns indicate the cold-test emergence of the seed, transferred 24 hours after planting to fresh soil.



GRAPH 9. Protecting value of the fungicide left in the soil from which the treated seed has been removed after 24 hours.

The protective value of the ceresan-new transplanted with the seed, decreased on all dosages used, as is indicated by the diminished percentage of germination in the cold-test.

The difference between the cold-test emergence of the non-transferred treated seed, which acts as a control in this case, and that of the transplanted seeds was 16.4, 29.0 and 29.2 %, for the dosages of 0.1, 0.2 and 0.3 % ceresan-new respectively.

In the case of arasan SF-X these differences are 8.3, 0.4 and 2.0 % respectively for the same dosages. Consequently only in the case of a dosage of 0.1 % of fungicide, is there an appreciable difference, whereas in the case of the heavier dosages the difference was negligible.

The conclusion is, that the thiram compound is more stable on the seed than the mercurial. This confirms the result obtained with the Glomerella agar test.

Because of the possibility that the ceresan-new which disappeared from the seed, might diffuse into the surrounding soil and sterilize it, non-treated seeds were planted in the holes from which the treated seeds had been removed. The percentage of cold-test germination of these fresh seeds indicates the protecting activity of the fungicide left in the soil, and is shown in Table 29 and in Graph 9. The result in this respect was opposite to what we expected. In the low dosage of 0.1 % neither ceresan-new nor arasan SF-X demonstrated a protecting activity in the surrounding soil; in the higher dosages the efficiency of the arasan in the surrounding soil is greater than that of the ceresan.

Consequently the amount of fungicide which disappeared from the seed was much greater in the case of ceresan, however, the resulting protecting activity of the surrounding soil was much less in the case of ceresan. This might be due to the fact, that the solubility of the organic mercurial compound, which is the active ingredient of ceresan-new, in the soil water is much greater than that of tetramethylthiaramdisulfide (the active ingredient of arasan), and therefore the mercurial in the surrounding soil was diluted to a level in which it is rarely toxic to the soil micro-organisms. Chemical decomposition of the mercurial is of course also a possibility. Arasan is very slightly soluble in water. It may for that reason be chemically more stable, and may consequently maintain its protecting activity.

CHAPTER IX

BEHAVIOUR OF FUNGICIDES AFTER SOIL TREATMENT

1. EFFECT OF SOIL TREATMENT WITH FUNGICIDES ON DAMPING-OFF OF CORN

In a preliminary experiment, naturally infested soil was treated with either arasan SF-X or ceresan-new in the standard rate of 0.1 gram per kg of soil. Non-treated seed was subjected to the cold-test in this soil. The following table shows the percentages of germination in this experiment.

TABLE 30. Cold-test emergence of corn in fungicide-treated soil

Soil treatment	Normal seedlings	Dead and abnormal	Percentage of germination
ceresan-new 0.1 %	143	7	95.3
arasan-SF-X 0.1 %	146	4	97.3
non-treated soil	16	134	10.6

Apparently the treatment of the soil with these two fungicides in the dosage used is very effective against the micro-organisms that cause damping-off of corn seedlings. The efficiency of both the fungicides is practically the same.

2. PERSISTENCE OF ARASAN SF-X AND OF CERESAN-NEW IN THE SOIL

The following experiment was undertaken to gain information concerning the time during which the fungicides arasan SF-X and ceresan-new persist in the soil in an active state. Naturally infested soil was treated with these fungicides in the rate of 0.1 g per kilogram of soil. The fungicide was well mixed with the soil by hand in wide trays. The soil was stored at room temperature in rectangular metal trays, which were enveloped in plastic sheets, permeable to air but not to water vapor, in order to keep the soil moisture at the original level. In order to exclude the possibility of a toxic effect of the zinc tray itself, it was also covered inside with plastic. Moreover the trays were weighed repeatedly during the period of storage, and water was added if required to maintain the original moisture content. The moisture in the soil was fixed in the beginning at 8.8 % by weight (water capacity of the soil used was 30.6 %). Immediately, and also after 1, 2, 4, 8, 14, 21, 28 and 42 days, one kilogram of soil was taken from the trays, and used for a cold-test with non-treated corn seed.

A comparable experiment was taken with the double dosage of the fungicides, and then after the same number of days, half a kilogram of soil was taken from the trays, mixed immediately with half a kilogram of fresh non-treated soil. The effect of this treatment was determined in a cold-test with non-treated corn seed.

The first experiment was conducted in order to determine the sterilizing effect of the treatment as well as its persistence. As the possibility existed that by the soil treatment the micro-organisms had been killed, the second experiment was taken with fresh micro-organisms added by means of an equal volume of fresh soil. In the first experiment the accumulative effect of the fungicide was determined, in the second the remaining activity.

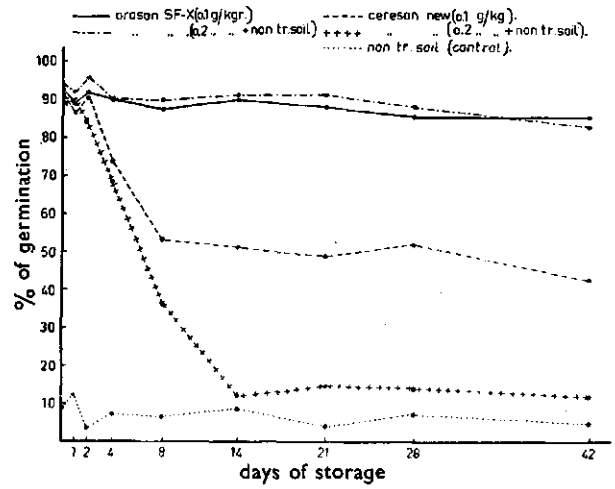
TABLE 31. Effect of arasan SF-X and ceresan-new in soil (0.1 g/kg) after different periods of storage – numbers indicate percentage of germination

Soil treatment	Storage periods in days								
	0	1	2	4	8	14	21	28	42
arasan SF-X	93.3	89.3	92.0	90.0	87.3	90.0	88.0	85.3	85.3
ceresan-new	91.3	86.6	90.6	74.0	53.3	61.6	48.6	52.0	42.6
non-treated soil	9.0	12.6	3.6	7.6	6.3	8.6	4.0	7.3	5.0

TABLE 32. Persistence of arasan SF-X and ceresan-new in soil (0.2 g/kg) after different periods of storage, mixed before testing with an equal volume of fresh soil – numbers indicate percentage of germination

Soil treatment	Storage periods in days								
	0	1	2	4	8	14	21	28	42
arasan SF-X	94.0	92.0	96.0	90.0	90.0	91.3	91.3	88.0	82.6
ceresan-new	88.6	89.3	84.0	67.3	36.6	12.0	14.6	10.6	8.6
non-treated soil	9.0	12.3	3.6	7.6	6.3	8.6	4.0	7.3	5.0

It is clear from Table 31 and the graph 10, that when the treated soil was tested as such for its cold-test value immediately, and also after 1 or 2 days of



GRAPH 10. Persistence of fungicide effect, and of fungicides in stored soil.

storing, that arasan SF-X and ceresan-new give a good control against damping-off of corn and increased the percentage of germination in the cold-test to the 90 % level. The effect of ceresan already begins to decrease after four days of storage, and gradually declines to an emergence of 42.6 % after 6 weeks. Arasan SF-X, on the other hand, practically maintains the original level of cold-test emergence, until the end of the experiment.

Table 32 and the graph 10 show that in the case of adding fresh micro-biologically active soil at the end of each storage period, the influence of ceresan-new as a protectant against damping-off of corn decreased much more rapidly, so that after two weeks storage its influence had practically disappeared. The activity of arasan SF-X in this experiment persisted much longer, so that only after six weeks a slight decrease in emergence became visible.

In both experiments the concentration of ceresan-new in the cold-test after two weeks storage was the same. In the second experiment the original fungicide concentration was twice that of the first, but this double concentration was halved before testing. In the first experiment the protecting activity of the ceresan-new appears to last much longer than two weeks, but that might be because in the beginning of the storage period the soil micro-organisms have been reduced to a very low level of activity, and have not yet recovered. According to MCKEEN (1949) (54) disturbance of the microbiological balance in the soil, may also result in protection against the soil microflora.

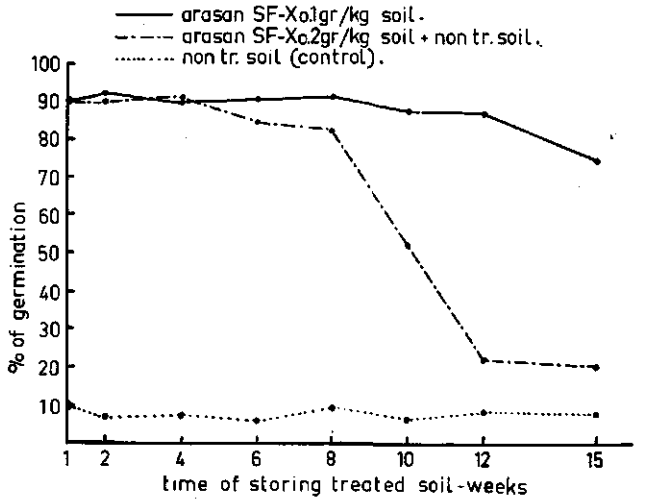
In the next experiment only arasan SF-X was used. This experiment was designed in order to find out how long arasan SF-X will stay active in the soil, and how long its effect will continue. The same thing for ceresan-new was already studied in the preceding experiments, but these experiments finished too early to study arasan, simultaneously. Again a large quantity of soil was treated with 0.1 gram of the fungicide per kilogram of soil, and stored in plastic-covered trays at room temperature. In order to exclude the possibility of a toxic effect of the zinc tray itself, it was also covered inside with plastic. Again water was added as required to maintain the original soil moisture content.

Soil samples from this tray were tested with the cold-test technique after 1, 2, 4, 6, 8, 10, 12 and 15 weeks of storage.

In addition, one tray was filled with soil, treated with 0.2 g/kg arasan SF-X, in which case the samples from the tray were diluted with an equal weight of non-treated natural soil before the test.

TABLE 33. Persistence and activity of arasan SF-X in treated soil – numbers indicate percentage of germination

Soil treatment	Weeks of storage							
	1	2	4	6	8	10	12	15
arasan SF-X 0.1 g/kg	90.6	92.6	98.3	90.0	91.3	86.6	86.6	74.0
arasan SF-X 0.2 g/kg + fr. soil	90.6	92.0	90.0	84.0	82.6	52.0	22.0	20.0
non-treated soil (fresh)	11.3	6.0	7.3	5.6	9.3	6.6	8.6	7.6



GRAPH 11. Persistence and activity of arasan SF-X in treated soil.

From this experiment it is clear that arasan SF-X showed a slight decrease, probably already after about 10 weeks, whereas it had undoubtedly progressed after 15 weeks. The first somewhat doubtful decrease was reflected in a reduction in cold-test value from about 90 to 86.6 %, the further decrease corresponded with a reduction in cold-test value to 74.0 %.

In the case of addition of fresh soil, in order to determine the influence of the remaining thiram on the soil microflora, a gradual and afterwards rapid deterioration was noted, as indicated by the cold-test figures of 84.0, 82.6, 52.0, 22.0 and 20.0 % after 6, 8, 10, 12 and 15 weeks of storage respectively.

CHAPTER X

EFFECT OF MOISTURE CONTENT OF THE SOIL, AND OF FUNGICIDE DOSAGE ON DAMPING-OFF OF CORN

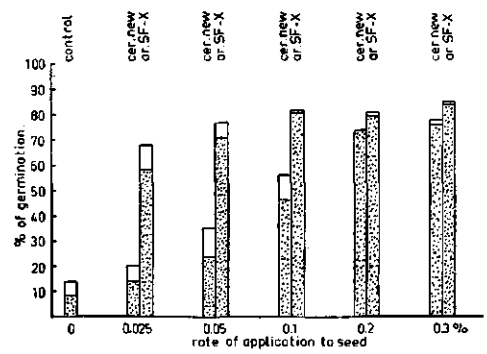
1. SEED TREATMENT

For the experiments described in this chapter, the same type of loamy sand was used as for the preceding experiments. This soil had a water capacity of 30.6 % (by weight) and was normally used with a water content of close to 9 %. In the following experiment, the water content was fixed at the levels of 25 % and 60 % respectively, of the water capacity. The fungicide dosage, of ceresan-new as well as of arasan SF-X, was 0.3, 0.2, 0.1, 0.05 and 0.025 % of the weight of the treated seed. In order to avoid loss of moisture, the trays were enveloped in plastic sheets during the seven days of low temperature; before the transfer to the 28 °C germinator the plastic sheets were removed.

The results of this experiments are given in the following table.

TABLE 34. Interaction of seed treatment and soil moisture on the cold-test emergence of corn – numbers indicate percentage of germination

Seed treatment and soil humidity	Fungicide dosage in %					
	0.3	0.2	0.1	0.05	0.025	control
ar. SF-X, moist. 25 %	85.6	80.8	82.1	77.6	69.6	13.6
ar. SF-X, moist. 60 %	84.0	79,4	81.3	71.3	59.0	7.3
cer-new, moist. 25 %	77.6	74.6	56.6	35.9	20.9	13.6
cer-new, moist. 60 %	76.0	74.6	46.6	24.3	14.3	7.6



GRAPH 12. Influence of seed treatment and soil humidity content on cold-test emergence of corn. Total height of the columns indicate emergence in soil with low humidity; dotted columns indicate emergence in soil with high humidity.

Thus in all treatments arasan SF-X proved to be better than ceresan-new. The efficiency of ceresan-new dropped much quicker than that of the thiram compound when the dosage was diminished. The percentage of cold-test germination, which indicates the efficiency of arasan SF-X, was about 85.6 and

84.0 % at the two humidity levels with the treatment dosage of 0.3 %, and dropped to 69.6 and 59.0 % respectively, when the dosage was decreased to 0.025 %.

In the case of the mercurial these percentages were 77.6 and 76.0 % at the highest dosage, and only 20.9 and 14.3 % at the lowest.

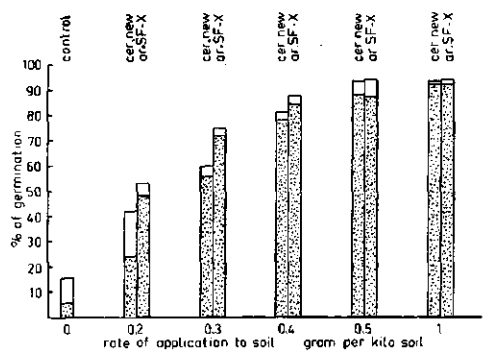
Consequently the soil humidity did not play a significant role when the fungicide dosage was normal, i.e. 0.3 or 0.2 %. Only at the very low dosages of 0.05 and 0.025 % in the case of arasan SF-X, and of 0.1, 0.05 and 0.025 % in the case of ceresan-new, and also in the control did the moisture level of the soil appear to be of influence on the cold-test emergence. This may be caused by the higher activity of the micro-organisms of the soil at the moisture level of about 60 %, as was suggested by REDDY & GERHOLD (63).

2. FUNGICIDE TREATMENT OF THE SOIL

To study the value of soil treatment, ceresan-new and arasan SF-X were used in dosages of 0.1, 0.05, 0.04, 0.03 and 0.02 g per kg of soil. The water content of the soil was fixed at two different levels, i.e. 25 and 60 %, respectively, of the saturation.

TABLE 35. Interaction of soil treatment and soil moisture on the cold-test emergence of corn – numbers indicate percentage of germination

Soil treatment and humidity	Fungicide dosage in g/kg soil					
	0.1	0.05	0.04	0.03	0.02	control
ar. SF-X, moist. 25 %	94.3	94.6	88.0	75.3	53.3	15.3
ar. SF-X, moist. 60 %	92.0	88.0	85.3	72.6	48.6	5.3
cer-new, moist. 25 %	93.6	94.0	81.3	60.6	42.6	15.3
cer-new, moist. 60 %	92.3	88.6	78.0	56.6	24.6	5.3



GRAPH 13. Influence of soil treatment and soil humidity on cold-test emergence of corn. Total height of the columns indicate emergence in soil with low humidity; dotted columns indicate emergence in soil with high humidity.

Both ceresan-new and arasan SF-X proved to offer good control against damping-off of corn when used in dosages of 0.1 and 0.05 g per kg of soil. When the fungicide dosage was decreased the efficiency of ceresan-new dropped much

quicker than that of arasan SF-X. This was indicated by the percentage of cold-test emergence, which in the case of arasan SF-X amounted to 94.3 and 92.0 % when the fungicide dosage was 0.1 g/kg and soil moisture 25 and 60 % of saturation respectively and dropped to 53.5 and 48.6 % when the dosage was decreased to 0.02 g/kg. In the case of ceresan-new these percentages were 93.6 and 92.3 % at the highest dosage, and decreased to 42.6 and 24.6 % at the lowest dosage.

Apparantly the soil moisture has a very slight effect in the case of arasan SF-X and in the case of ceresan-new when used in the higher dosages. Only in the lowest dosage of ceresan-new used, that of 0.02 g/kg, the difference was great. Also in the control it was very important. It is possible that when the dosage of ceresan-new is much decreased, this fungicide is diluted by the soil water, so that when much water is present a level is reached in which the fungicide is no longer toxic to the soil fungi.

DISCUSSION

The present study was performed with the purpose of investigating the relative value, and comparing the mode of action of organic mercury compounds and thiram when used for seed treatment. Former workers have demonstrated that one may distinguish between the disinfectant activity and the protectant activity of the seed dressing. In modern scientific language the term "protection" is used in another meaning than was formerly done. In the older literature this term was used to indicate the activity of fungicides in general, including their action on seed-borne infections (MARTIN 53, HORSFALL 37). At present, workers prefer to use the word "disinfection" for the combating of seed-borne diseases whereas the term "protection" in relation to seeds is reserved for the control of soil-borne fungi, which may attack seed and seedlings. In this sense the terms are used in the present publication, which has for its purpose the study of the disinfectant and protectant activity of different fungicides when used as seed dressings and when applied to the soil.

For studying the disinfectant activity of fungicides, seed with a more or less deep-seated infection may be investigated in germination experiments in a virtually sterile medium.The choice fell on radish seed with Alternaria infection, which was studied mainly in the blotter test. Heavily infected radish seed could easily be obtained; the infection is known from literature as being difficult to control by chemical means (GROVES & SKOLKO, 28); it is easily detected in a germination test of short duration on moist filterpaper.

For Alternaria-infected radish seed, mercury compounds gave better results than thiram, and of the mercurial treatments tried ceresan-wet proved to be superior. This, of course, is in agreement with the general opinion, which holds that the more volatile mercury compounds penetrate more deeply into the seed, and consequently perform their work better than non-volatile compounds. Later the study of disinfectant activity was restricted to the ceresan-wet treatment.

By means of sections it was proved, that most of the infection is present in the surface layers of the seed coat, part of it has penetrated into the deeper layers of the seed coat, and some seeds appear to have infected the cotyledons and the germ. The author's standard treatment with 1 % ceresan-wet for 8 minutes proved to be a practical method for controlling the infection including

the deep-seated fungus. However, when after this treatment the seed coat of the non-germinated seeds was removed, some of the deep-seated infection appeared to be viable. These non-germinated seeds are the heavily infected ones. The fungicide on their surface prevents the appearance of the fungus, but when the seed coat and with it the fungicide, is removed, the survival of the infection becomes apparent.

When treated seed was stored for some weeks in a closed bottle, the fungicide succeeded in killing all infection. However, the germinating capacity of the seed was also severely impaired in that case.

Finally a number of antibiotics were tried for *Alternaria* in radish seed. Only rimocidin, when used as 200 p.p.m. solution for 16 hours, succeeded in controlling the superficial infection. For practical purposes this would be sufficient, as is ceresan-wet at 1 % for 8 minutes. When used at 400 p.p.m. for 30 hours rimocidin also killed the deep-seated fungus without causing injury to the seed, and is therefore superior to the ceresan-wet treatment plus storage.

For studying the protectant activity of fungicides the choice of a suitable kind of seed, and a reliable method is more difficult. Seed is needed with a low resistance against adverse conditions, e.g. against the natural soil fungi under such conditions. The method must be conditioned so that duplicable results may be obtained. The author succeeded in obtaining sensitive samples of corn seed, for which species a suitable testing technique is available in the American cold-test. In this technique the weak seeds, untreated or after fungicide treatment, are confronted with the omnipresent semi-parasitical soil flora under adverse conditions for the seed. The resulting emergence percentages, can be taken as a measure for the protective value of the seed dressing.

By means of the cold-test, thiram proved to afford a far better protection for corn against soil-borne fungi, than the mercury compounds. Afterwards the author restricted himself to comparing the action of ceresan-new (as a representative of the mercurial group) and arasan SF-X, because thiram is generally considered as a typical protectant fungicide.

Thiram persists much longer than the mercury compound on the treated seed, as well as in treated soil. This persistence is of great practical importance, because in field sowings it is seldom known when the adverse conditions will start and how long they will continue.

In storage experiments, seeds treated with ceresan-new started with a rather low cold-test emergence figure, reaching a maximum after about 1 day storage. This is probably because the fungicide needs some time for a strong adsorption to the seed coat. After about a month a very great part of its effect was lost, and after two months it had totally disappeared. Thiram persists much longer on the seed, but even here some decrease became visible after 6 weeks; after about 12 weeks this decrease had become very clear and the same level was maintained till the end of the experiment after 28 weeks.

It appeared that ceresan-new was also quickly lost from the treated seed after sowing, without affording a strong protective action to the soil surrounding the seed. Arasan persists much longer on the planted seed, and even the small quantity which is lost to the surrounding soil causes an appreciable reduction in damping-off potency of this soil.

When used for treating the soil with the standard dosage of 0.1 g per kg, both ceresan-new and arasan SF-X were very effective in reducing damping-off of corn in the cold-test. In low concentrations ceresan-new partly failed,

especially when the soil humidity was high. Moreover in storage experiments with treated soil, the influence of thiram persisted much longer than that of ceresan-new. The former maintained its full protective value for about 12 weeks, the latter already showed a decline after 4 days. Yet after these 12 weeks storage the thiram has also largely disappeared. This was demonstrated by adding fresh natural soil at the end of the storage period, by which measure the thiram effect appeared to be very much reduced. Apparently the microflora in the thiram-treated soil is slow in recovering, and even when the fungicide itself has largely disappeared its effect persists. This might be due either to a partial sterilization of the soil, or to disturbance of the natural microbiological equilibrium.

The influence of soil humidity was not important, between reasonable limits, for the emergence of the treated seed, or the emergence of non-treated seed in treated soil, was scarcely changed by an increase of the soil moisture from 25 to 60 % of the saturation. This seems to be in contradiction with the opinion of REDDY & GERHOLD (63), but probably the result of the present study may be considered an extreme case, caused by the use of an extremely weak and sensitive seed lot. Only with very low fungicide dosages to the seed or in the soil, as well as with untreated seed and soil, the influence of soil moisture became clearly visible. This might explain the fact, that throughout this study emergence percentages of the non-treated control fluctuated much more than those of treated corn seed.

SUMMARY

Part I

The results obtained can be summarized as follows.

Organic mercury compounds proved to be more effective for combating the Alternaria infection in radish seed than thiram products (Table 2).

Dust treatments with mercury products, did not result, however, in complete control of the infection, not even when used in dosages up to 10 g per kg seed (Tables 4 and 5).

Wet treatments with ceresan or germisan gave the best results. The best dust treatment – ceresan-dry – gave about 84 % reduction in disease, whereas ceresan-wet decreased the infection with about 99 %.

Wet treatments with thiram were not better than dust treatments with the same compound (Table 3).

Although wet treatments with mercury compounds may also endanger the seed, ceresan-wet and germisan-wet in a concentration of 1 % for 4 to 8 minutes, still had no deleterious effect on the viability of radish seed (Table 6).

On the other hand this dosage resulted in nearly complete control of the disease (Table 7).

In section 2 of the third chapter it is demonstrated that the Alternaria infection may be present in every part of the seed, and that in the sample used for these experiments, in about 1 in 5 infected seeds, the fungus had penetrated into the interior of the seed (Tables 8 and 9).

These results were confirmed by making microtome sections of infected seeds and staining them. The hyphae of the pathogen were found in the seed coat

cells, in the open space between the seed coat and the underlying cotyledon, and also in the intercellular spaces of the outer cotyledon as well as of the hypocotyl (see photographs).

The seeds with deep-seated infection are among those that in the germination test fail to germinate (Table 9).

By means of the Glomerella-agar test it was found that the inhibition zone around seeds treated with ceresan-wet, is larger than that around seeds treated with ceresan-dry. This means that after the wet treatment the seed carries more mercury than after the dust treatment (photo 8).

Also with this method it was demonstrated that ceresan-wet does not penetrate through the seed coat into the interior of the seed (photo 9).

Furthermore it appeared that washing after the treatment largely removes the ceresan-dry, but only partially removes the fungicide from the seeds after a wet treatment (photo 9).

Table 10 proves that ceresan-wet is unable to kill the fungus situated in the interior of the radish seed. This is in agreement with the results of the Glomerella test. The deeply-infected seeds do not germinate in the blotter test.

After treatment with ceresan-wet and a subsequent germination period of 7 days, the fungus can still be present in a living state within non-germinated seeds. The deep-seated infections are partly killed during and after the treatment and partly survive but cannot develop unless the seed coat is removed from those seeds (Table 11).

From Table 12 it appears that the effect of the fungicide is accumulated during storage after treatment, so that after two weeks the seed is quite free from the infection. However, in this period the germinating capacity of the seed is severely impaired.

The antibiotics agrimycin, aureomycin, terramycin, penicillin and vengicide had no effect on the Alternaria infection of radish seed (Table 13).

Actidione in a concentration of 100 p.p.m. for 8 hours is sufficient for controlling the disease, but a the same time is phytotoxic to the seed (Table 14).

Rimocidin offers better possibilities, as in dosages of 200 p.p.m. for 16 hours, 400 ppm. for 8 hours, and 800 p.p.m. for 4 hours it succeeds in completely controlling the infection without any deleterious effect on the seed viability (Tables 15 and 16).

Also rimocidin does not succeed in killing the fungus in the interior of the seed immediately after the treatment, although rimocidin 200 p.p.m. during 18 hours is slightly superior to ceresan-wet 1 % for 6 minutes (without storage) for combating the infection (Table 17).

Although rimocidin 200 p.p.m. for 18 hours did not kill all of the Alternaria infection immediately after application, it did succeed in doing so during the subsequent germination test, whereas ceresan-wet did not obtain the same result (Table 18).

Soaking the seed in 400 p.p.m. for 30–40 hours is sufficient for killing all of the infection immediately. The same result is obtained after treatment with 1 % ceresan-wet for 8 minutes, followed by two weeks storage. This rimocidine treatment, however, is not phytotoxic to the seed, whereas the ceresan-wet treatment becomes phytotoxic during the storage period (Table 19).

Part II

In the cold-test thiram products proved superior to mercury compounds as protectant fungicides for corn against the soil micro-organisms that cause damping-off.

Arasan SF-X was the most effective of the thiram materials tried (Table 22).

Arasan SF-X gave better protection to corn seed than ceresan-new in all the dosages used.

When these two fungicides were applied in the normal dosages of 0.2 or 0.3 %, the differences in efficiency were smaller than when they were used in higher or lower dosages.

Ceresan-new proved to be phytotoxic to corn when used in the high dosages of 0.4 and 0.8 % (Tables 23 and 24, Graphs 3 and 4).

By means of the Glomerella agar test, it was proved that arasan SF-X persists much longer than ceresan-new when the treated seed was stored in open dishes at room temperature. Ceresan-new decreased gradually and disappeared within 60 days; with arasan SF-X the size of the fungus-free zones showed a decrease after 60 days (Table 25, Graph 5).

This result was confirmed by the cold-test technique (Table 26, Graph 6).

After treatment with 0.2 % arasan SF-X the decrease in efficiency in the cold-test appeared to become visible after six weeks, progressing slowly and gradually till the end of the experiment (from about 80 % at the beginning to 48 % after 28 weeks; Table 27, Graph 7).

The Glomerella agar test was also used to demonstrate that the thiram, after planting the seed in soil, persists longer on the seed than does the mercury compound. With arasan SF-X the inhibition zone did not shrink till 20 days after planting; with ceresan-new the decrease already became visible after 1 and 2 days, whereas it disappeared within 4 days (Table 28).

The decline in protective value of the fungicides on treated seed in the soil was also investigated with the cold-test technique. Arasan SF-X adheres strongly to the seed, so that when the treated seed is transplanted after 24 hours to fresh soil the protection is still excellent. Ceresan-new is not so stable on the seed; depending on the fungicide dosage used, a great part of the protective activity is not transmitted with the seed to fresh soil. On the other hand the protective value of the soil, that has been in contact with treated seed and is afterwards used for the sowing of fresh seeds, is greater in the case of arasan SF-X than it is in the case of ceresan-new. With low seed treatment dosages there was no protective activity communicated to the soil, but with higher dosages it became important especially with the thiram (Table 29, Graph 8).

When used for treating the soil both the fungicides were very effective when used in the normal dosages of 0.1 g/kg soil (Table 30).

In storage experiments with treated soil arasan SF-X showed very little decline in effect during a seven week period at room temperature; ceresan-new, however, started to lose its effect already after 4 days (Table 31, Graph 10). When inoculum was added in the form of natural soil at the end of the storage period, the protection by ceresan-new declined still faster and practically disappeared in the course of two weeks, whereas arasan SF-X in this case only lost very little of its activity after six weeks storage (Table 32, Graph 10). In an experiment of 15 weeks duration arasan SF-X still maintained the greater part of its protection when no fresh soil was added, but when after the storage

period fresh soil was added, the protection was largely lost (Table 33, Graph 11). In studying the influence of soil moisture, it appeared that with thiram-treated seed this influence was negligible when the normal dosage of 0.2 or 0.3 % was used, but in the lower treatment dosages and for non-treated seed a higher water content of the soil resulted in a lower cold-test emergence. The same is true for ceresan-new, which, however, soon failed in the lower dosages (Table 34, Graph 12). The influence of soil moisture also was not important for the emergence of non-treated seed in treated soil (Table 35, Graph 13).

SAMENVATTING

DEEL I

DE AANWEZIGHEID VAN *ALTERNARIA SPP.* IN RADIJSZAAD EN ZIJN BESTRIJDING MET CHEMISCHE MIDDELEN

HOOFDSTUK I

Relatieve werkzaamheid van verschillende fungiciden

Organische kwikprodukten zijn gebleken effectiever te zijn ter bestrijding van de Alternaria-infectie van radijszaad dan thiramprodukten (tabel 2). Droogbehandeling met kwikverbindingen bleek echter onvoldoende om de infectie geheel te onderdrukken, zelfs niet bij doseringen tot 10 gram per kg zaad (tabellen 4 en 5). Natbehandeling met ceresan of germisan gaf de beste resultaten. Door de beste droogbehandeling – met ceresan-droog – werd de infectie met omstreeks 84 % verminderd; met ceresan-nat bleef slechts ongeveer 1 % der oorspronkelijke infectie over. Natbehandeling met thiram bleek niet beter dan droogbehandeling met dezelfde verbinding (tabel 3).

HOOFDSTUK II

Toepassing van natontsmetting met kwikverbindingen

Hoewel natbehandeling met kwikverbindingen schadelijk kan zijn voor het zaad, bleken ceresan-nat en germisan-nat, toegepast in een concentratie van 1 % gedurende 4 tot 8 minuten, de kiemkracht van het zaad nog niet te verminderen (tabel 6). Anderzijds bleek deze dosering de ziekte vrijwel geheel te onderdrukken (tabel 7).

HOOFDSTUK III

Lokalisatie van Alternaria *in het zaad*

In dit hoofdstuk wordt aangetoond, dat de Alternaria-infectie kan zetelen in alle delen van het zaad. In het voor het onderzoek gebruikte zaadmonster was ongeveer een vijfde van de zaden diep geinfecteerd (tabellen 8 en 9). Deze vondst werd bevestigd door onderzoek van gekleurde coupes van zaden. De hyphen van de schimmel werden aangetoond in de cellen van de zaadhuid, in de ruimte tussen de zaadhuid en de buitenste zaadlob, en ook in de intercellulaire ruimten van het buitenste cotyl en van het hypocotyl (zie de foto's). Niet alle zaden met diepzittende infectie bleken in de filtreerpapier-kiemproef te kiemen (tabel 9).

HOOFDSTUK IV

Werkingswijze van Ceresan-nat

Door middel van de Glomerella-agarproef werd aangetoond, dat de remmingszone rondom de met ceresan-nat behandelde zaden groter is dan die rondom met ceresan-droog behandelde zaden. Dit toont aan, dat het zaad door de natbehandeling meer kwik meekrijgt dan door de droogbehandeling (foto 8). Ook werd met deze methode bewezen, dat het ceresan-nat niet doordringt tot in het binnenste van het zaad (foto 9). Verder bleek door wassen na de behandeling ceresan-droog grotendeels teverdwijnen, terwijl na nat-behandeling het fungicide maar gedeeltelijk door wassen te verwijderen bleek (foto 9).

Uit tabel 10 blijkt, dat ceresan-nat de schimmel binnenin de zaden niet doodt. Dit komt overeen met de resultaten van de Glomerella-agarproef. De diepgeïnfecteerde zaden kiemen echter niet op vochtig filtreerpapier en blijven na ontsmetting uitwendig vrij van schimmel. Na behandeling met ceresan-nat en een verblijf van 7 dagen in het kiembed kan de schimmel nog levend binnenin ongekiemde zaden aanwezig zijn. De diepzittende infecties worden gedurende en na de fungicide-behandeling gedeeltelijk gedood, gedeeltelijk blijven ze in leven zonder dat de schimmel te voorschijn kan komen, tenzij de zaadhuid van de betreffende zaden wordt verwijderd (tabel 11).

Uit tabel 12 blijkt, dat het effect van de fungicide-behandeling toeneemt tijdens bewaring na de behandeling, zodat na twee weken de infectie geheel gedood is. Gedurende die tijd wordt echter ook het zaad zelf ernstig beschadigd.

HOOFDSTUK V

Gebruik van antibiotica bij de bestrijding

De antibiotica agrimycine, aureomycine, terramycine, penicilline en vengicide bleken geen effect te hebben op de Alternaria-besmetting van radijszaad (tabel 13). Rimocidine biedt gunstiger mogelijkheden, want met doseringen van 200 p.p.m. gedurende 16 uur, 400 p.p.m. gedurende 8 uur, en 800 p.p.m. gedurende 4 uur werd de infectie geheel onderdrukt zonder dat het zaad zelf beschadigd werd (tabellen 15 en 16). Ook rimocidine doodt echter de inwendige infectie niet tijdens of direct na de behandeling. Wel werkt rimocidine 200 p.p.m./18 uur iets gunstiger dan ceresan-nat 1 %/6 minuten zonder bewaring (zie tabel 17). Hoewel dus rimocidine 200 p.p.m./18 uur niet voldoende bleek om de Alternaria-infectie geheel te doden direct na de behandeling, bleek dit doel wel te worden bereikt tijdens de op de behandeling volgende kiemproef. Met ceresan-nat bleek hetzelfde niet te worden bereikt (tabel 18). Door rimocidine 400 p.p.m. gedurende 30 tot 40 uur wordt de infectie wel geheel gedood. Na behandeling met ceresan-nat in concentratie 1% gedurende 8 minuten is daarvoor nog twee weken bewaring van het behandelde zaad nodig. Deze rimocidine-behandeling is echter niet toxisch, de voor de ontsmetting gelijkwaardige ceresan-behandeling plus bewaring wel (tabel 19).

DEEL II

DE RELATIEVE WAARDE EN DE WERKINGSWIJZE VAN THIRAM EN KWIKVERBINDINGEN TOEGEPAST ALS ZAADBESCHERMERS TEGEN BODEMSCHIMMELS

HOOFDSTUK VI

Inleiding

De literatuur wordt besproken en de werkwijze beschreven

HOOFDSTUK VII

Relatieve werkzaamheid van verschillende fungiciden tegen wegvallen van maiskiemplanten

In de cold-test voor mais bleken thiramprodukten beter te beschermen tegen de kiemrot veroorzakende grondorganismen dan kwikverbindingen. Van de onderzochte thiramprodukten bleek arasan SF-X het gunstigst te werken (tabel 22). Dit produkt bleek maiszaad beter te beschermen na uitzaai dan ceresan-nieuw in de beproefde doseringen. Als de genoemde fungiciden in de normale doses van 2 tot 3 gram per kg zaad werden toegepast waren de verschillen in werking kleiner dan als ze in hogere of lagere doseringen werden gebruikt. In de hoge doses van 4 tot 8 g/kg bleek ceresan-nieuw phytotoxisch voor mais (tabellen 23 en 24, grafieken 3 en 4).

HOOFDSTUK VIII

Vergelijking van de duurwerking (persistentie) van arasan SF-X en ceresan-nieuw op maiskorrels

Met de Glomerella-agarproef werd aangetoond, dat bij bewaring van behandeld zaad in open Petrischalen bij kamertemperatuur arasan SF-X veel minder snel verdwijnt dan ceresan-nieuw. Ceresan-nieuw verminderde geleidelijk in hoeveelheid en was na 60 dagen geheel verdwenen; met arasan SF-X vertoonde de schimmelvrije zone pas na 60 dagen een duidelijke vermindering in grootte (tabel 25, grafiek 5). Deze resultaten vonden bevestiging in coldtestproeven (tabel 26, grafiek 6). Na behandeling met arasan SF-X 0.2 % werd de vermindering in beschermende werking in de coldtest zichtbaar na zes weken en nam langzaam toe tot aan het einde van de proef (coldtest-opkomst bij het begin der proef 80 %, na 28 weken nog 48 %. Tabel 27, grafiek 7).

De Glomerella-agarproef werd eveneens gebruikt om aan te tonen, dat thiram na het zaaien langer op het zaad aanwezig blijft dan de kwikverbinding. Na behandeling van het zaad met arasan SF-X werd de remmingszone pas kleiner 20 dagen na de zaai; met ceresan-nieuw werd hij 1 tot 2 dagen na de zaai al kleiner, terwijl 4 dagen na de zaai de kwikverbinding niet meer aantoonbaar was (tabel 28). De vermindering van de beschermende werking van de fungiciden na uitzaai van het behandelde zaad in grond werd ook gevonden met de coldtest-methode. Arasan SF-X blijft sterk aan het zaad hechten, zodat als na 24 uur het behandelde zaad wordt opgegraven en overgebracht in verse grond de bescherming nog uitstekend is. Ceresan-nieuw handhaaft zich niet zo goed op het zaad: in afhankelijkheid van de dosering van het produkt wordt een meer of minder groot gedeelte van de beschermende werking niet mee overgebracht met het zaad naar de verse grond. Anderzijds blijkt de beschermende werking van de

grond, die deze gekregen heeft door contact met behandeld zaad, groter in geval van arasan SF-X dan in geval van ceresan-nieuw. Dit werd aangetoond door in die grond nieuwe, onbehandelde zaden te zaaien. Na behandeling van het zaad met lage doseringen fungicide verkreeg de grond helemaal geen beschermende werkzaamheid; na behandeling met hogere doseringen bleek de beschermende werking van de grond vooral groot als thiram gebruikt was (tabel 29, grafiek 8).

HOOFDSTUK IX

Werkzaamheid van fungiciden na behandeling van de grond

Bij gebruik als grondontsmetters bleken beide fungiciden zeer effectief als ze werden toegepast in de normale dosering van 0.1 gram per kg grond (tabel 30). In proeven met behandelde grond vertoonde arasan SF-X geringe achteruitgang in werkzaamheid gedurende zeven weken bewaring bij kamertemperatuur; ceresan-nieuw bleek al na 4 dagen te verminderen (tabel 31, grafiek 10). Als de behandelde grond aan het einde van de bewaarperiode voorzien werd van nieuw inoculum door vermenging met verse grond, dan bleek de beschermende werking door ceresan-nieuw nog sneller achteruit te gaan en in de loop van twee weken praktisch geheel verdwenen te zijn. Arasan SF-X bleek onder gelijke omstandigheden in zes weken nog maar weinig aan werking verloren te hebben (tabel 32, grafiek 10). Zelfs in een 15 weken durende proef bleek arasan SF-X zijn werkzaamheid grotendeels te hebben behouden als geen verse grond werd toegevoegd, maar als na die tijd verse grond werd toegevoegd bleef van de beschermende werking weinig over (tabel 33, grafiek 11).

HOOFDSTUK X

Effect van de vochtigheid van de grond en van de hoeveelheid fungicide op het wegvallen van maiskiemplanten

Bij bestudering van de invloed der grondvochtigheid bleek, dat na behandeling van het zaad met thiram in de normale doseringen van 2 à 3 gram per kg de grondvochtigheid van geringe invloed was op de coldtest-opkomst; bij lage doseringen echter, en vooral bij gebruik van onbehandeld zaad veroorzaakte een hoger watergehalte van de grond een lagere opkomst in de coldtest. Hetzelfde geldt voor ceresan-nieuw, dat echter in lagere dosering eerder faalt (tabel 34, grafiek 12). Ook bleek de invloed der grondvochtigheid niet groot als onbehandeld zaad in behandelde grond werd gezaaid (tabel 35, grafiek 13).

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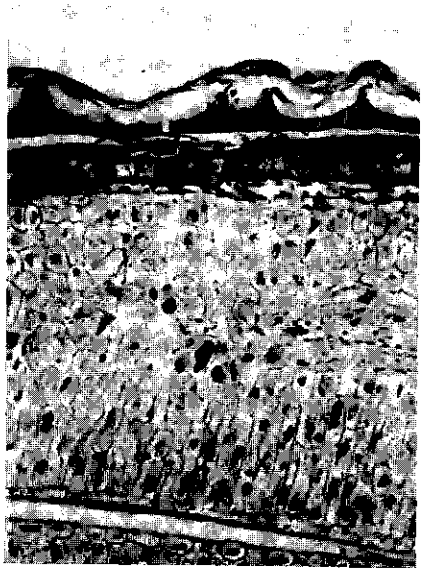
PHOTOGRAPHS

1. Section of a non-infected radish seed, showing the way which the cotyledons envelop the axis (radicle + plumule). The seed coat is made up of some layers of different types of cells.
2. Low power magnification of the seed coat of an infected seed.
3. High power magnification of part of photo 2, with the dark hyphae visible within the seed coat cells, and also in the open space between the seed coat and the underlaying cotyledon tissue.
4. This shows the extent of the invasion by the Alternaria mycelium through the intercellulars of the outer cotyledon, coming from the direction of the seed coat and penetrating to a depth of many cell layers.
5. High power magnification of the Alternaria mycelium within the intercellular spaces of the cotyledon close to the seed coat in the upper part of photo 4.

PLATE 1

1

2



3

4

5

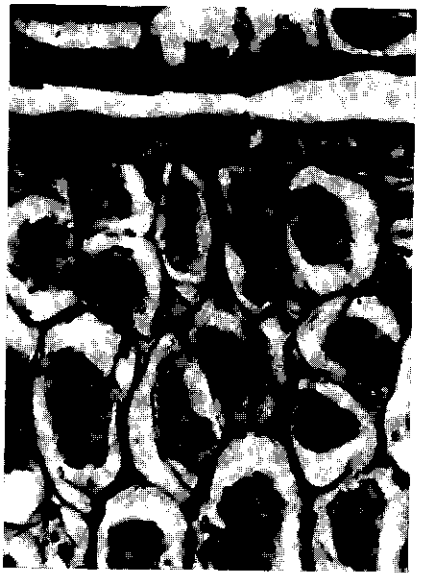
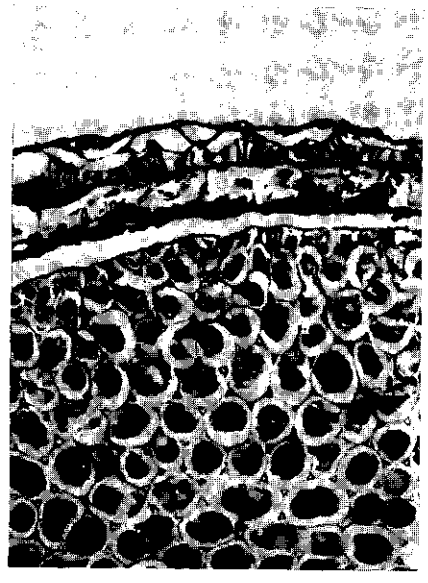
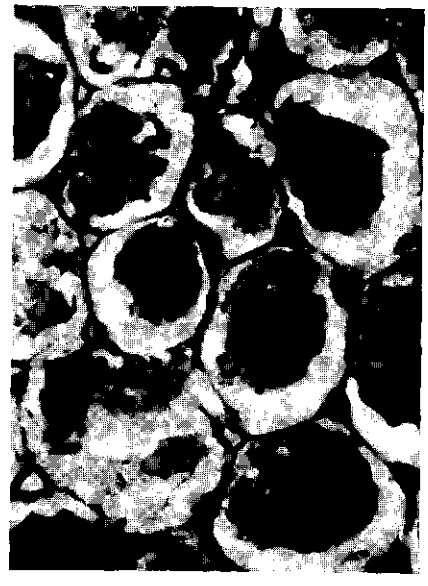


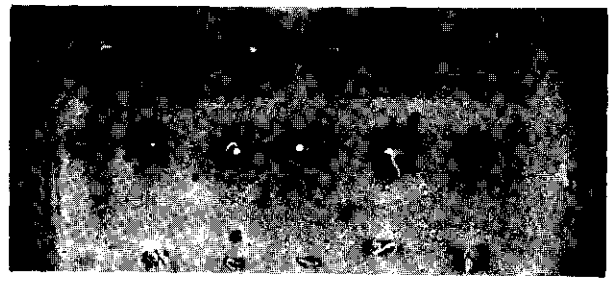
PLATE 2



6



7



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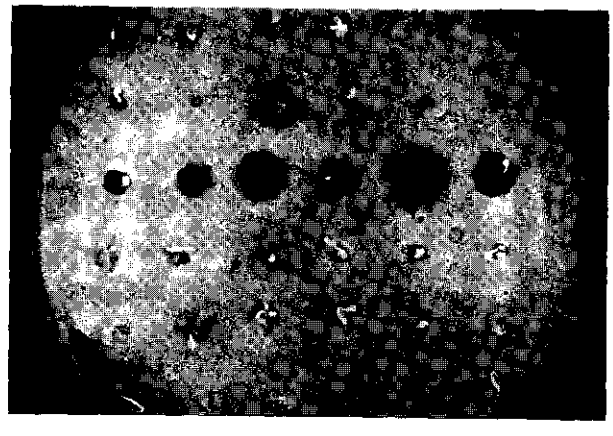

9

6. In this part of the section of the radish seed, naturally infected with *Alternaria spp.*, the mycelium in the intercellular spaces of the axis may be seen, penetrating until the seventh cell layer.
7. High magnification of part of the axis, with the mycelium completely filling some of the intercellular spaces and partly filling others. The septated hyphae are clearly visible in some places.
8. Inhibition zones obtained by means of Glomerella agar test using seeds treated with ceresan wet (1 % solution at 4–8 minutes) and ceresan-new (dry).
9. Shows the inhibition zones obtained by using the following treatment:
   Row no. 1 shows the non-treated seed (no inhibition zone);
   Row no. 2 seeds treated with ceresan new and afterwards washed. Here is no inhibition zone, so that the fungicide dust apparently is easily removed by washing;
   Row no. 3 – The inhibition zone demonstrates that after wet treatment and washing, at least a great part of the fungicide is still present on the seeds.
   Rows no. 4, 5 and 6 give a repetition of the experiment, but with the seed coats taken off before planting the seeds. No zone is obtained anywhere.