INJURY BY ORGANIC MERCURY SEED TREATMENT IN PEAS

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THESIS

This is to certify that the

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John R. Vaughan
Major professor

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INJURY BY ORGANIC MERCURY SEED TREATMENT IN PEAS

By

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INTRODUCTION

Seed treatment occupies a prominent place in agriculture because the first step towards growing a good crop is to sow healthy seeds. Four kinds of seed treatment have been recognized according to their nature and their purpose: (1) Seed disinfection aims to eradicate the fungus or bacterium which has infected the seed and is established within the seed coat or in deeper-seated tissue. (2) Seed disinfestation destroys the microorganisms on the seed coat. (3) Seed protection, as the name implies, is intended to protect the seeds from the attacks of rotting organisms. (4) Seed inoculation with nitrogen-fixing bacteria, though not a recent development, has become more and more important in field legumes.

The most prominent type of treatment is seed protection, because it is easier to protect than to cure. This involves at the present time the use of fungicides, either in a dust form or semiliquid paste, which are applied to the seed coat. Before the advent of the nonmetallic seed protectants, the poisonous and volatile organic mercury dusts were prominent in the field of seed treatment. Although injury from such dusts has been known to occur under excessive moisture, poor ventilation and high temperature, they have been more effective in cereal disease control than any other type of fungicide. A slurry method of treating seeds has been devised to prevent the worker from inhaling the dusts and to apply more efficaciously the fungicide onto the seed coat and the dust containing a wettable carrier adheres more tightly to it. However, because of the moisture involved, chemical injury is more likely to occur. Serious injury following such a method has been reported on peanuts (55) and on peas (13). Such injury and inconvenience partly impairs benefits gained from the slurry method. Little is known concerning the mechanism by which the slurry method injures the seeds, and there is some question as to when injury to the embryo occurs. Extensive histological and cytological studies have been made on corn seedlings poisoned with ethyl mercury phosphate (46), but no reports of such studies can be found in the literature on dicotyledonous plants. For these reasons, an investigation was begun in the spring of 1951 to study the mechanism by which the slurry method of seed treatment causes injury to peas. Certain anatomical and histological deformations resulting from organic mercury treatment were studied.

REVIEW OF LITERATURE

History of Organic Mercurials

About 1900, European scientists found that certain organic mercury compounds were highly efficient in destroying bacteria and their spores (16). Their results led to further investigations on the use of these materials. According to Gabel (17), mention of organic mercurial salts in seed treatment was first made in 1913. The simplest organic compound used, cyanide of mercury, was reported to be completely effective in preventing stripe disease of barley when used as a steep for the grain. Mercury chlorophenol, introduced by Remi in Germany for the steeping of cereals attacked by Fusarium, was the chief component of the fungicide "Uspulum." Another organic salt of mercury, mercury cresol sodium cyanide, was the chief component of the fungicide "Germisan." These fungicides were introduced in the United States about 1925 (20), and soon were followed by other new organic mercurials. Of these newer fungicides, the organic mercurials known to have been injurious under various conditions are listed in Table 1.

Table 1. Organic mercurials occasionally injurious to seeds.

Trade Name	Composition	Company
Semesan	30% hydroxymercury chlorophenol	E. I. duPont de Nemours, Wilmington, Del.
Semesan Bel	12% hydroxymercury nitrophenol and hydroxymercury chlorophenol	E. I. duPont de Nemours, Wilmington, Del.
Ceresan	2% ethyl mercury chloride	E. I. duPont de Nemours, Wilmington, Del.
Ceresan M	7.7% ethyl mercury p-toluene sulfonanilide	E. I. duPont de Nemours, Wilmington, Del.
New Improved Ceresan	5% ethyl mercury phosphate	Bayer Semesan Div. of E. I. duPont de Nemours, Wilmington, Del.

Several causes of poor emergence of seedlings could be seed decay by rotting organisms and injury by toxic seed protectants. Injury by organic mercurials, though not always detected, may often contribute to the delay emergence. Numerous reports of visible toxicity by the organic mercurials to seeds and seedlings of cereals, ornamentals, field legumes and vegetables can be found in the literature.

Injury to Cereals

When correctly used, organic-mercury seed treatments give an excellent control of many seed-borne diseases, but under some conditions injury to the grains results and considerable loss has been reported. Weston and Brett (54) recorded some factors which predispose the grain to injury such as excessive moisture, high temperature and poor ventilation. Crosier (10) reported abnormal germination in a sample of Marquis spring wheat, which was treated with Ceresan and stored before planting. Records of disappointing field stands have been common. When treated with dry dusts, the seeds germinated well, but when treated with Ceresan while moist, they produced roots and plumules that failed to elongate normally and were abnormally thickened. Porter (45) reported in 1936 that cereal seeds treated with an overdose (any amount in excess of that required for protection) of mercurials had thickened leaf primordia with irregular crenations and lobes. Cell division was inhibited and the existing cells had become enlarged and multinucleate, either with small nuclei or with large polyploid "giant nuclei." Atkins and Stamps (2) mentioned in 1948 that seed treatments on oats infected with Helminthosporium victoriae had not given good results. Some of the mercurials drastically reduced stands when used in excess. In 1951 Craley and French (8) found that increasing rates of Ceresan M from 3/4 to 1-1/2 ounce per bushel progressively injured rice seedlings. Stephenson (47) reported that grass seeds were killed if soaked in a saturated solution of Semesan, Semesan Bel, or Ceresan. Hoppe (23) reported that seeds mechanically injured along the edges of the embryo developed the stunted and swollen condition typical of mercury poisoning.

Injury to Ornamentals

Person and Chilton (43) found that Ceresan, Semesan, New Improved Ceresan and Semesan Jr. injured ornamental seeds when applied in full strength. Gladiolus corms are known to absorb toxic amounts of mercury from mercuric chloride (41) and are injured by organic mercurials such as Semesan (21). There was a direct relationship between external injury and mercury content in Narcissus bulbs treated with New Improved Ceresan, Ceresan M, and 2 percent Ceresan reported by McClellan (33). Gould and Miller (20) reported that phenyl mercury acetate (1 pound to 700 gallons of water) used as a dip was much safer than the standard 2 percent Ceresan dip, even though some bulbs were injured.

Injury to Miscellaneous Crops

Thomas (48) treated safflower seeds with Ceresan M at the rate of 0.5 ounce per bushel in an attempt to control rust (Puccinia carthami). He found partial control and some reduction in stand at 9 ounces per bushel, the maximum dosage that the seed would hold. Ciferri (6) reported in 1951 that an experimental mercurial ''zeolite'' used as a tobacco seed dressing caused primary lesions of the phloem. The hypocotyl was stunted, twisted, and hypertrophied. Xylem cells contiguous to the phloem were interrupted, and endodermal as well as cortical cells were collapsed.

Injury to Field Legumes

The literature concerning the effects of chemical treatment on nodulation has been conflicting. Appleman (1) found satisfactory nodulation on peas and soybeans grown from inoculated seed treated with Semesan. In his tests Ceresan prevented nodulation on canning peas, but not on soybeans. In the latter case all nodulations appeared to be on lateral roots and not the taproot, as typically obtained on plants from nontreated inoculated seeds. Kadow, Allison, and Anderson (29) found a decrease of nodulation from an average of 75 nodules (per plant nontreated) to 3 or 4 nodules per plant as a result of treating with an unidentified organic mercury. Some of the earlier work by Miller and Stapp reported by Appleman (1) demonstrated that if nodule bacteria are in the soil at the time of planting, seed treatments do not hinder the development of root nodules. In trials testing the importance of seed inoculating with a bacterial inoculum such as "Nitragin," Vlitos and Preston (50) excluded Ceresan M because in preliminary work this fungicide reduced considerably the stands of Austrian winter pea, mung-bean chinese red cowpea, yellow hop clover and hairy wetch. Recently Gederman (19) studied the effects of Arasan, Phygon, and Ceresan M on red clover, Alfafa, and sweet clover in wet and dry soil. In general the crops were benefited by most treatments. Phygon and Arasan, nonmercury compounds which appeared harmless in wet soil, reduced the emergence or injured the seedlings when the seed was planted in dry soil. In greenhouse tests with 1 percent Ceresan M, red clover failed to form roots and there was a swelling of the hypocotyl. Seedlings of alfalfa and sweet clover were only slightly affected by the same treatment.

Injury to Vegetables Other than Peas

Bald (3) reported that mercury treatments were toxic to the cells of potato tubers and probably also to the rotting organisms. For this reason, when used to treat tubers, organic mercury dips gave some protection to the sets cut from them, without noticeably affecting suberization. Its effects on the cut surfaces, were however to destroy many layers of cells, and absorption of mercury seriously affected the emergence and subsequent growth of the potato shoots. Muller (34), as reported by Clayton (7), found "Germisan" and "Uspulum" toxic to tomato and celery seed in the concentration usually employed. Horsfall (24), in 1930, found that Semesan was highly injurious to tomatoes under certain conditions "not well understood as yet." Clayton (7) treated tomato seeds with a liquid organic mercurial and found germination temporarily inhibited. He concluded that it was safer in this case to use organic mercury dusts. Vaughan (49) reported that concentrations of ethyl mercury phosphate greater than 1/20,000 used on tomato seed caused a reduction in the percentage of germination as well as a slowing up of the rate of germination. Davis and Haenseler (12) however, found no visible injury with New Improved Ceresan to tomato in the soil greenhouse tests, but a slight injury with New Improved Ceresan in sand tests. Leach (30), in tests combining fungicidal treatments with pelleting materials, remarked that the delay in emergence of pelleted tomato seeds, previously treated with mercuric chloride or New Improved Ceresan dip, was greater than the additive delays from coating and treating separately. The inclusion of organic mercury compounds in coating resulted in reduced and retarded emergence.

Miller and Grogan (35) treated tomato seed with mercuric chloride (1/3,000) and with New Improved Ceresan (1/1,200). They concluded that when the ratio of seed weight to the volume of treating solution was increased above 1:8, germination was impaired. Dickey and Ark (14, 15) investigated injury to tomato seeds treated with mercurials. They determined the location and the concentration of the mercury within the seeds by using the dithizone method (35). Seeds treated with mercuric chloride (1:1000) for 10 minutes and washed in water for 15 minutes were germinated in petri dishes and in pots in the greenhouse. No trace of mercury was detected at any time in the embryo after this treatment. On the sixth day after planting, the mercury in the remaining parts of the seed showed a uniform lowering of concentration to 30 to 35 percent of the amount present immediately after treatment. The endosperm, with the inner cellular layer attached, contained 14 to 18 percent as much mercury as the seed coat. The critical amount of mercury in the endosperm of nongerminated seeds was shown to be 0.6 to 0.7 p.p.m. Treated seeds were germinated in pots in the greenhouse receiving definite amounts of water each day. In both loam and sandy soil a relationship was found between the amount of mercury removed, the amount of water supplied and germination of seeds. These authors studied the penetration of mercury into tomato seeds and found it to be proportional to the logarithm of the treatment time. Penetration was more rapid in the case of mercuric chloride and proceeded at an even rate from the epidermis to the embryo.

Dried shell and snap beans are known to be only slightly susceptible to mercury injury in common practice even though often overdosed. However, snap beans (43) germinated poorly when soaked in a solution of 1:1500 mercuric chloride in 70 percent alcohol plus 2 percent acetic acid to control bacterial blight. No report has been found on injury by organic mercury compounds.

Lima beans are known to be very sensitive to mercury injury. Clayton (7) observed in 1931 that after two weeks of growth, plants from nontreated seeds were bigger than the plants from Semesan treated seeds. His photographs showed the stunting effect of the plants as well as the poor development of the root system. New Improved Ceresan also injured lima beans (11, 37).

Peanut seed has been reported to be affected by Ceresan M (55). As the dosage of Ceresan M was increased, the emergence also increased reaching a peak at 1-1/2 to 3 ounces per 100 pounds of seed. At 4-1/2 ounces per 100 pounds, the emergence was less than that for the untreated seeds. Occasionally Ceresan M was found to be toxic to peanut seed even at 3 ounces per 100 pounds of seed. In this case the primary root of the seedling typically grew for an inch or slightly more, but the epicotyl did not develop. The hypocotyl continued to increase in diameter and resulted in a deformed seedling.

Injury to Peas

Jones (27) was the first to report application of organic mercurials on peas. Haenseler (22) found a decrease in germination of pea seed after liquid Semesan treatment. He attributed however the decrease in germination to a mechanical injury resulting from handling the swollen pea rather than injury to the germs by chemicals. Crosier and Patrick (10) mentioned injury in peas caused by New Improved Ceresan. McNew (38, 39, 40) showed that ethyl mercury phosphate retarded plant growth of Green Admiral, Wisconsin, and Surprise peas and in addition reduced the yield of Surprise variety. He concluded that fungicides other than organic mercurials should be used on peas. Walker et al. (53) found in 1940 that Ceresan was not as effective as were the common copper oxide seed treatments. Walker suggested that low emergences from these treatments might have been due either to low protective values or to injurious effects of the fungicide. In 1941 New Improved Ceresan was found to be injurious to peas in New York State (42). Hull (25) noticed that the tap roots of Alaska pea seedlings were noticeably more stunted by heavy dosage of a dry organic mercury seed dressing than were those of Gradus. It was found by deZeeuw and Andersen (13) that response of pea varieties to Ceresan M varied with the method of applying fungicide to the seed. Dry applications of 4 ounces per 100 pounds of seed resulted in significant stand increases, whereas stands of many varieties were reduced significantly with the same rate of application of the fungicide in water slurry.

MATERIALS AND METHODS

The effects of mercury poisoning on peas by organic mercury slurries were studied first in the field, then in the laboratory. Anatomical and histological studies were made on three of the pea varieties, Alaska wilt resistant, Dwarf Gray Sugar and Wisconsin Perfection; which deZeeuw and Andersen (13) found to respond differently when treated with Ceresan M. Alaska was injured severely, Dwarf Gray Sugar slightly, and Wisconsin Perfection was ordinarily not injured at the rate they used.

In addition to Ceresan M, another organic mercurial, Agrox, whose active ingredient is phenyl mercury urea, and mercuric chloride were included in the field tests to see whether or not they would cause a similar injury. Phygon Xl, a non-metallic and nontoxic fungicide, which had been shown to be effective in pea protection, was used as a control in order to determine whether poor emergence, if any, was due to the effects of rotting organisms or to the toxicity of the materials.

All these seed protectants were applied to the seeds by the slurry method. As has been mentioned above, the slurry method of treating seeds was devised to eliminate dust in the atmosphere and to provide a more uniform and accurate application of the fungicide on the seed coat. Because of the small amount of seeds used in these experiments the slurry method was modified as follows:

Six hundred seeds were accurately weighed in order to determine the required amount of fungicide (4 ounces per 100 pounds or 0.0025 of the seed weight). This amount of fungicide was mixed with 5 drops of water or less in a beaker. The percentage of moisture of this semipaste is very important, for too much or too little of water will prevent the even coating of the seeds. Only by trial and error the quantity of water can be determined. However, the best method was found to be a rotating of the beaker containing the seeds and the fungicide in semipaste form and to add if necessary one drop of water at a time until no residual dust remained on the side of the vessel. After treatment the peas were dried thoroughly before being germinated in the field or in the laboratory.

In the field experiment, pea seeds of the three varieties were treated with Ceresan M and Agrox at the rate of 2 ounces and 4 ounces per 100 pounds, with Phygon Xl at the rate of 4 ounces per 100 pounds and with mercuric chloride (1:1000 solution) at the rate of 5 cubic centimeters per 100 seeds. The field layout was a split plot design. Because one of the main objects was to determine varietal differences in peas to the seed protectants, the smallest split in the plot design was the varieties within each treatment. Each row was 25 feet long, spaced 28 inches apart and 100 seeds were planted in a row with a V-belt planter. Stand counts, height measurements and observations of any visible injury were recorded.

In the laboratory seeds were treated either with Ceresan M at the rate of 4 ounces per 100 pounds of seed or with an overdose of wet Ceresan M. Such treated seeds and nontreated (control) seeds were germinated in wet rolled paper towels put sidewise in covered jars so that the lower ends of the towels were in an inch of water at the bottom. Roots from treated and untreated peas were killed in a standard strong Formalin Aceto-Alcohol solution, dehydrated in a standard tertiary butyl alcohol series and embedded in paraffin (26). Sections were made at 10 /u and 30 /u and stained by the Foster tannic acid method (16). Measurements of cell and nucleus size were made with a calibrated ocular micrometer. The smear technique was used to study polyploidy in root cells. Stains used to color the nuclei included Fuelgen, aceto-carmine and propionic acid.

EXPERIMENTAL RESULTS

Field Experiment

The seeds were treated on August 20, 1951, planted three days later, and seedling counts made on September 4. Table 2 shows the data from these counts together with the average stands for the various treatments.

Analysis of the stand data showed that Ceresan M used at the rate of four ounces per 100 pounds of seed in slurry form was injurious to all three varieties (average stands of 23.5, 39.5, and 50, as compared to Phygon XI with 89, 61.7, and 87.51). No varietal difference could be found in this respect. Agrox at both rates was a good seed fungicide and mercuric chloride was of no value in controlling damping off. Inspection in the field of the growing plants in the 4 ounces Ceresan M rows disclosed that they were stunted. Some varietal difference could be found. Alaska peas were the most injured, Dwarf Gray sugar less, and Wisconsin Perfection the least. The average height of the plants from the various treatments taken from one representative replication is shown in Table 3. Data

Table 2. Stand counts of three varieties of peas treated with four chemicals.

Treatments and Rate	Replications				Total Plants	Avg. No. of Plants
	I	II	III	IV		
Alaska Wilt Resistant						
Ceresan M - 2 oz.	a/72	64	78	91	305	76.5
Ceresan M - 4 oz.	13	39	12	29	93	23.5
Phygon Xl - 4 oz.	86	89	91	90	356	89
Agrox - 2 oz.	84	91	86	80	341	85
Agrox - 4 oz.	85	74	66	88	313	78
1/1,000 $HgCl_2$ - 5 cc.	58	52	74	63	247	61.7
Check (no treatment)	37	46	47	59	189	47.5
Wisconsin Perfection						
Ceresan M - 2 oz.	65	71	56	59	251	62.7
Ceresan M - 4 oz.	25	56	30	37	158	39.5
Phygon Xl - 4 oz.	58	70	54	65	247	61.7
Agrox - 2 oz.	46	53	46	37	182	45.5
Agrox - 4 oz.	55	65	57	73	244	61
1/1,000 $HgCl_2$ - 5 cc.	4	7	5	3	19	4.75
Check (no treatment)	5	4	1	7	17	4.25

Table 2 (Continued)

Treatments and Rate	Replications				Total Plants	Avg. No. of Plants
	I	II	III	IV		
			Dwarf Gray Sugar			
Ceresan M - 2 oz.	94	92	93	99	378	94.5
Ceresan M - 4 oz.	50	59	50	42	201	50
Phygon Xl - 4 oz.	72	87	93	99	351	87.75
Agrox - 2 oz.	100	87	96	99	382	95.5
Agrox - 4 oz.	88	89	95	95	357	91.5
1/1,000 $HgCl_2$ - 5 cc.	97	91	79	99	362	90.5
Check (no treatment)	93	99	94	93	379	94.7

a. 100 seeds planted per row.

Interpretation:

L.S.D. for treatment within each variety at 5 percent level - 10.8.

L.S.D. for treatment within each variety at 1 percent level - 13.8.

Table 3. Average height of three pea varieties grown from seeds treated with various seed treatment fungicides.*

Slurry Treatment and Rate	Dwarf Gray Sugar	Wisconsin Perfection	Alaska Wilt Resistant
Ceresan M - 2 oz.	9.1 cm.	7.1 cm.	12.3 cm.
Ceresan M - 4 oz.	6.4	4.8	3.6
Phygon Xl - 4 oz.	9.1	9.6	16.0
Agrox - 2 oz.	9.4	8.3	13.9
Agrox - 4 oz.	9.3	6.8	13.3
1/1,000 $HgCl_2$ - 5 cc. for 150 seeds	9.3	5.05	13.4
Check	9.3	5	16
Average	9.3	6.66	12.63

* Plants from one representative replication.

observations show that the height of the three pea varieties was severely reduced by Ceresan M at the rate of four ounces per 100 pounds. The height of the Dwarf Gray Sugar peas, a variety resistant to damping off, seemed to be unaffected by seed treatment with Agrox, mercuric chloride, or Phygon Xl. On the other hand better results were obtained with the Alaska and Wisconsin Perfection varieties when their seeds were treated with Phygon Xl and the plants were more vigorous.

Representative plants from rows treated with Ceresan M at four ounces were carefully dug and the roots examined for signs of mercury injury. The photographs (Plates I, II, and III) taken three weeks after planting illustrate the typical symptoms in each case. The roots of affected plants were shortened and bronzed at the tip; the hypocotyl was greatly enlarged and the stem was apparently normal, although it was short in proportion to the root system. However, as the photographs show, by the end of the season the plants had grown adventitious roots above the cotyledons. These started to elongate and allow partial recovery but the cold weather stopped any additional growth that might have occurred.

Effect of Storage on Organic Mercury Treated Seed

Chemically treated seeds may be injured under various environmental conditions during storage, such as high temperature, high humidity, and lack of aeration. According to Leach *et al.* (29), addition of 10 percent moisture to sugar beet seeds treated with Ceresan and subsequently stored for 10 days before planting, retarded germination and produced stunted seedlings. Addition of 6 percent moisture and confinement in a dry space for 30 days resulted in injury to part of the seedlings, whereas 2 percent moisture was noninjurious. Baylis (4) treated samples of wheat seed with Agrosan G. and Ceresan U. T. 1875 A. at dosages of 2 and 4 ounces per bushel, and stored them for 12 months. The moisture content of the seed samples ranged from 12.8 to 16.7 percent. In general the lower the moisture content of the seed, the higher was the field germination of both treated and untreated seeds. Brett and Weston (5) dusted seeds of wheat, oats, and barley with organic mercury protectants and found that seeds of good quality and proper moisture content were not injured after one year storage.

Because of the high moisture content of the seed following the slurry method of seed treatment used in these trials, the question arises as to when the mercurial injury occurs. Is it immediately after treatment and during storage, or is it during germination? It has been proved by Wain (49) that in the case of copper seed treatments in peas, the injury occurred during germination. The theory was that pea seeds, when they germinate, render soluble the insoluble copper complex ion, which in turn injures the embryo after passage through the micropyle and the seed coat. It was thought that mercury might injure in a similar manner during germination. To test this hypothesis, seeds which had been treated with an excess of wet Ceresan M and stored for a month were divided into two lots. The first lot had their seed coats removed immediately after a 25 minute soaking in water. The second lot was soaked for 25 minutes, but their seed coats were not removed. Both lots were germinated and the seedlings obtained were compared to seedlings from nontreated seeds. Results showed that seedlings obtained from treated seeds with seed coats removed germinated as well as those from nontreated seeds, though the roots were slightly injured and curved. The lot of treated seeds with seed coats left on was completely inhibited. This indicated that injury to peas by mercury is more likely to occur during germination.

Histological Studies

As stated above, the germination of peas treated with an overdose of wet Ceresan M was completely inhibited. Such seeds had necrotic spots visible on their seed coats (Plate IV, Fig. D.). When they were split for examination the tip of the radicle and the testa were particularly darkened.

Nontreated seeds produced seedlings with straight thin roots (Plate IV, Fig. A). Such roots seen in longitudinal section had regular polyhedral cells. These cells from the tip of the meristematic tissue to the beginning of the differentiation layer, were nearly square: 25 to 30 /u in diameter, and their nuclei were always present and occupied nearly half the cell with a diameter of 10.8 /u to 13.5 /u, giving a ratio, nucleus to cell, of 1/2. Cross sections made in the beginning of the differentiation layer showed that these cells were usually octogonal (Plate IV, Fig. F).

Seeds treated with Ceresan M at the rate of 4 ounces per 100 pounds produced seedlings with slightly to seriously injured enlarged roots. The tip of these roots was generally necrotic and seen in longitudinal section had distorted cells (Plate IV, Fig. B and C). The cell wall instead of being a straight line, curved in and out and often seemed to be collapsed. Except for the epidermal cells there was no uniformity in cell size. These distorted cells were greatly enlarged in comparison to normal cells, being at least 50 /u in diameter. When the roots were cut at 10 /u the cells contained few nuclei. Table 4 gives an average of 20 measurements of abnormal root cell diameter and nucleus diameter. The ratio of nucleus diameter to cell diameter was 0.16, and showed to be inferior to the one in normal cells 1/2. This gave an indication that the cell enlarged more than the nucleus. When seen in cross-section, the cells seemed less distorted (Plate IV, Fig. H), but they were twice as large as normal cells and less distinctly octogonal.

The average number of cells across the width of an abnormal root was found to be 17, as compared to 21 from the same region of a normal root. This indicated that the

Table 4. Twenty measurements of root cell and nucleus diameter from peas treated with Ceresan M at the rate of four ounces per one hundred pounds and from non-treated peas.

	Abnormal		Normal	
	Cell Diameter	Nucleus Diameter	Cell Diameter	Nucleus Diameter
	81 /u	18.9	27	13
	121.5	24.3	27	13
	67.5	10.8	27	13
	135	27	27	13
	108	18.9	27	13
	76.5	8.1	27	13
	67.5	13.5	27	13
	108	16.2	27	13
	135	27	27	13
	94.5	16.9	27	13
	81	13.5	27	13
	108	16.2	27	13
	140	40	27	13
	135	27	27	13
	140	26	27	13
	80	8.1	27	13
	108	15.9	27	13
	63.5	16.2	27	13
	110	16.2	27	13
Total	2042	327.4		
Average	10.2	16.2	27	13
Ratio: Nucleus/Cell	0.16		0.49	

obvious swelling of the abnormal roots was due to the enlargement of the individual cells.

Shoots from peas that had been treated with Ceresan M in excess were cross-sectioned. No visible injury could be found in the shoot cells which appeared normal.

PLATE I

Injury of Alaska Wilt Resistant pea caused by an organic mercury seed treatment material, Ceresan M.

Left: Control, nontreated.

Right: Treated with Ceresan M in aqueous slurry at the rate of 4 ounces per 100 pounds of seed.


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PLATE II

Injury of Wisconsin Perfection caused by an organic mercury seed treatment material, Ceresan M.

Left: Control, nontreated.

Right: Treated with Ceresan M in aqueous slurry at the rate of 4 ounces per 100 pounds of seed.


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PLATE III

Injury of Dwarf Gray Sugar by an organic mercury seed treatment material, Ceresan M.

Left: Control, nontreated.

Right: Treated with Ceresan M at the rate of 4 ounces per 100 pounds of seed.


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PLATE IV

Anatomical aspects of uninjured pea seedlings and those injured by mercury containing seed treatments.

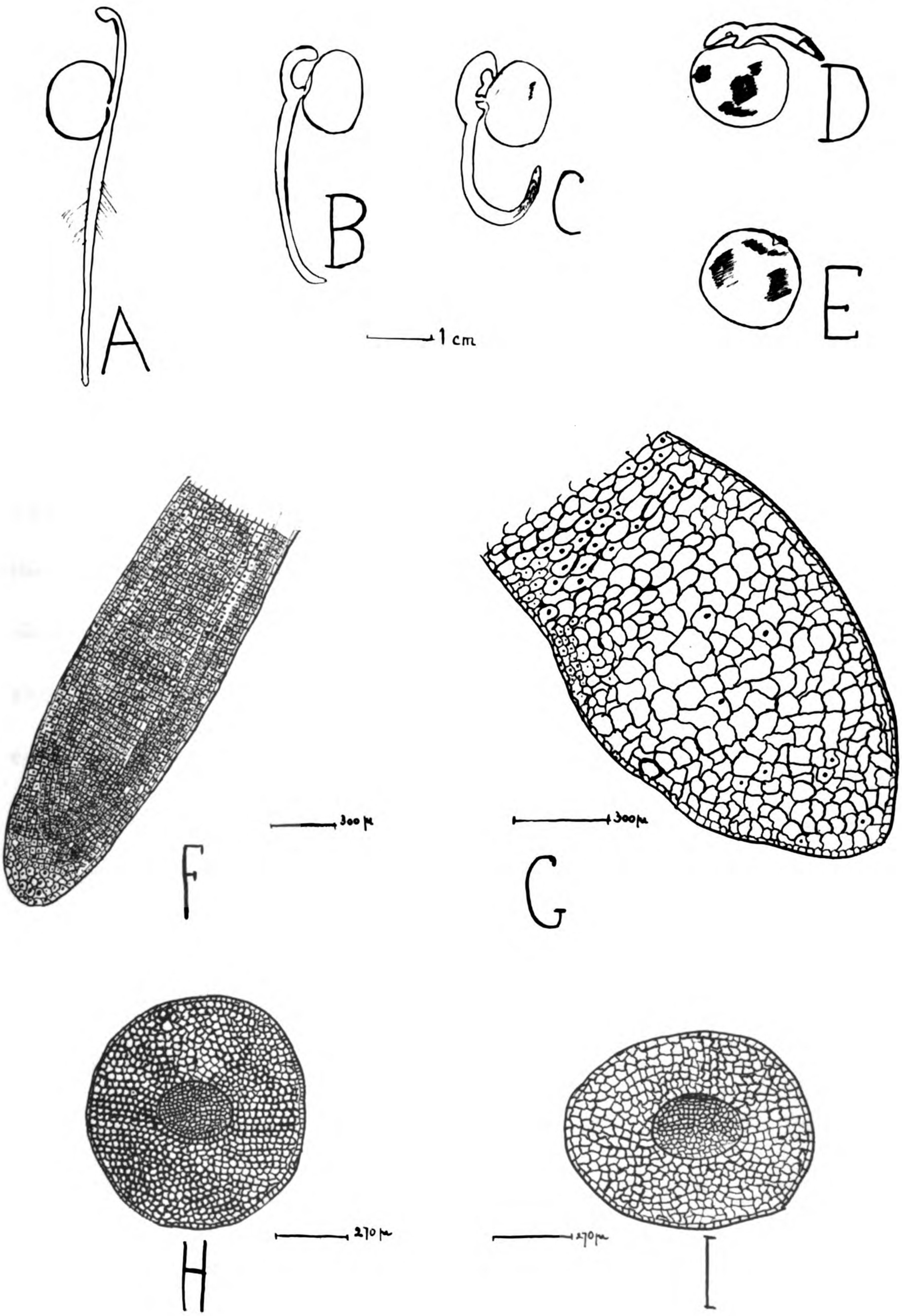
A. Normal seedling.

B, C, D. Slight to serious injury.

E. Complete inhibition.

F, G. Longitudinal sections of normal and abnormal pea roots.

H, I. Cross sections of normal and abnormal pea roots at the beginning of the differentiation layer.


A
B
C
D
E
1 cm
F
300 μ
G
300 μ
H
270 μ
I
270 μ

DISCUSSION

The results obtained in this investigation are similar to those obtained by others (10, 13, 45, 50, 55) on cereals, clover, peanuts, and peas. Mercurial toxicity resulted in an inhibition in length of the developing primary root and in a thickening of the root due to an enlargement of the cells. In peas, the stem was not directly affected by mercurial poisoning as shown by the presence of adventitious roots from the epicotyl. This field observation was confirmed in the laboratory by cross-sectioning shoot tips of pea seedlings. No gross anatomical differences could be found between abnormal and normal seedlings. This is of importance because according to Sass (46), the plumules of corn as well as the roots were injured by organic mercurials. No reason can be given on the strength of this work as to why pea stems were noninjured by Ceresan M and further work should be done along this line.

The field results were conclusive and confirmed that Ceresan M was injurious to the three pea varieties. However, some varietal difference could be found in response to this fungicide. Differences in seed coat structure or in the physiology of these varieties may be the answer. Besides direct observations of mercurial injury to peas, it should be noted that Phygon Xl was found to be a very good seed protectant for Alaska and Wisconsin Perfection. Dwarf Gray Sugar was in these tests a variety resistant to damping off as shown by the stand data of the control plants.

In all histological preparations made, the cells had lost their meristematic aspect. No mitosis was seen. Figure G, Plate IV, showed no differentiation of plerone and periblem layers. The question arose is this a median longitudinal section. For if it is not a median longitudinal section, this differentiation would not be seen. Because of the bending effect that the root takes after treatment, straight sections through the differentiation layers are hard to obtain. From observations of histological preparations the root appeared to have started differentiation but had stopped. More histological preparations should be made to understand how the plerone and periblem layers are affected by mercury.

Porter (45) reported that the cells of abnormal roots injured by an overdosage of New Improved Ceresan became multinucleate. This was confirmed by Sass (44) on the basis of extensive histological and cytological studies on the malformation of corn seedlings treated with a 1 to 1:1500 solution of New Improved Ceresan. The seedlings exhibited various degrees of distortion of cells, tissues, and organs in proportion to the severity of the gross external symptoms. "Isolated areas of enlarged cells were found to occur in the older leaf primordia of the plumule. Hypertrophy progressed from the end of the primordium and spread to the apical meristem. These cells were binucleate." Because of these findings the writer looked for multinucleate or giant nucleate cells for confirmation. He was unable to find multinucleate cells by any of the common techniques. They did not appear in any of the preparations of root tip smears or in the smears of more mature tissues. Nor was the writer successful in finding them in paraffin sections. This does not mean that Ceresan M under certain conditions cannot cause polyploidy or polyteny (or both) by failure of mitosis thus resulting in giant nucleate cells or multinucleate cells. In fact, the organic mercury dusts which are known to cause these abnormalities have to be used in overdose (45, 18). One of the difficulties encountered was that pea seeds treated with an overdose of wet Ceresan M were totally inhibited in their germination and roots from such seedlings are naturally unavailable. All the work then had to be done with peas in such a way that injury would be manifested without producing inhibition. Sass (44), in his cytological studies, used corn roots from seeds already germinated and subsequently treated with a solution of New Improved Ceresan. If such a method were used with pea roots, it should be possible to ascertain whether or not Ceresan M causes polyploidy. Gassner (17) in 1950 reported that overdosages of nonsubstituted aliphatic mercury compounds caused polyploidy. Since Ceresan M is such a compound, it would be worthwhile to do further work. It was found that Agrox did not cause any injury to the three pea varieties which confirms Gassner's statement that nonsubstituted aromatic compounds (of the type of Agrox) are nontoxic.

As stated above, there was a slight injury on seeds treated with an overdose of wet Ceresan, when the seed coat had been removed. These results were only an indication that injury is likely to occur during germination. Because there was a slight injury, mercury in some form could have penetrated into the seeds during storage. McCoach (34) described an analytical method for determining minute quantities of organic mercury materials in plant tissues. The organic mercury was converted to mercury dithizonate and was extracted with carbon tetrachloride. Determination of the mercury content was made by measuring the density of the extract against a reference solution with a spectrophotometer. Such a method by which mercury could be determined in or under the seed coat before and after storage would be very useful in determining the time when injury occurs. In storage tests it should be possible to find whether or not toxic quantities of mercury move to the embryo when the seed is dry or whether injury begins at germination.

SUMMARY

Organic mercury protectants were applied to three varieties of peas in field and laboratory tests to study the anatomical and histological deformations resulting from mercury injury.

A field experiment showed that Ceresan M slurry at the rate of 4 ounces per 100 pounds of seed affected in varying degrees the three varieties of peas tested. Alaska was visibly more injured than Dwarf Gray Sugar and Wisconsin Perfection. Agrox did not affect the same varieties under the same conditions.

Symptoms of mercurial poisoning on the pea are stunting of the upper part of the plants due to an inhibition of the primary root, enlargement of the hypocotyl and secondary formation of adventitious roots from the epicotyl. The adventitious roots may allow the plant to recover partially from previous root injury but the plants never attained the vigor of normal ones.

Histological studies showed that the enlargement of the hypocotyl as well of the primary root was due to an enlargement

of the existing cells. These cells were distorted and apparently had stopped mitosis. There appeared to be a correlation between the size of cell and size of the nucleus. However, this trend towards polyploidy or polyteny was not confirmed by any of the common cytological methods.

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