THE DAMPING-OFF OF TOBACCO AND ITS CONTROL IN PUERTO RICO [1]

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In recent years there has been great alarm among the tobacco growers of Puerto Rico because of the spread of a persistent malady in our tobacco seed-beds, the damping-off disease. The studies begun six years ago are outlined and discussed in this paper. The chief aim has been to develop a practical working method for the control of this dreaded malady.

Damping-off is here treated chiefly in relation to tobacco (*Nicotiana tabacum L.*). We have shown that the pathogenes responsible for damping-off of tobacco may also produce a similar disease in some garden vegetable and castor bean seedlings.

NAMES

The most common name for the disease in Puerto Rico is "sancocho" or "salcocho." Recently, the names "salcocho blanco" (white damping-off) and "salcocho prieto" (black damping-off), have been introduced into the long list of popular agricultural names, to distinguish between what the growers regard as two forms of the disease.

HISTORY AND RANGE

Hesse's investigations in 1874 (16) mark the beginning of numerous researches on damping-off on many of the higher plants. Most of the literature on the subject, however, deals with the disease on suscepts other than tobacco. The disease probably occurred on tobacco as early as it did on the other suscepts. Breda de Haan (5) reported a seed-bed rot of tobacco from Sumatra and Java in 1896. Cook and Horne (9) reported damping-off from Cuba in 1905. The disease has subsequently been the subject of special studies there. Search among publications where tobacco in Puerto Rico is mentioned has shown that, apparently, the first report of the disease in that Island is by Loew (20) in 1908. To quote: "a peculiar disease, spreading from a center in ring-like progression, was observed in the

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tobacco seed-beds of Caguas. The circle of dead bleached seedlings increased continuously and could be checked only by removing the soil to some depth and treating the spot with diluted formaldehyde (1 spoonful to 30 gallons of water). The disease is called "sancochado" in Porto Rico."

It has been reported from various other parts of the world. In Puerto Rico it has been prevalent probably during the last three or four decades but its importance has been largely disregarded. Damping-off, in general, was briefly discussed by Johnson (19) in 1914, who placed special emphasis on the subject of the control in relation to tobacco.

This malady now occurs in both tropical and temperate zones but its severity is increased as tropical conditions are approached. In some countries soil sterilization is now a common practice and methods of culture are so advanced that the disease is rarely serious.

It is present in nearly all the tobacco soils of the true tobacco sections of Puerto Rico. One of the pathogenes, *Phytophthora Parasitica** has not been found in the coastal section from Arecibo to Aguadilla where poorer, native types of tobacco are grown, but the other, *Pythium debaryanum,* is common. Here the temperature is relatively higher and the humidity lower than in higher elevations.

Importance

The losses from damping-off fluctuate from year to year. In certain seasons when precipitation is moderate, the disease occurs only in localized areas in the seed-beds and may be easily kept in check. On the other hand, should heavy rains be prevalent throughout a season, the magnitude of the losses will be very great. During the season of 1927, heavy rains brought about a disastrous condition. Late plantings had to be made and there were not enough seedlings for a normal planting. In many instances the seedlings were completely swept away by the disease. Reports from various sections of the island and the author's own observations showed losses from 8 per cent to as high as 90 per cent. Data obtained from the agricultural agents on about 105 acres of cloth-shaded beds and 310 acres of open beds in 1927, showed a range of infection of from 8 to 80 per cent. If a curve were to be plotted with these data, the mean would be above 50 per cent and the mode would exceed that figure. In 1928 the losses were quite severe in some sections while in 1929 the season was especially unfavorable for the disease.

* Throughout this paper the designation *P. Parasitica* stands for *Phytophthora Parasitica* Dastur, var. *nicotianae* Tucker.

Under such circumstances tobacco planting, in certain years, is quite an uncertain proposition. Even when growers plant in advance to allow a wide margin for losses from damping-off, the extent of his planting remains uncertain until the seed-bed season is nearing the end. The disease may destroy the larger part of his seedlings and then he will be at the mercy of the other planters whose seedlings may be of undesirable varieties or of poor quality. If he succeeds in buying enough seedlings for his farm he will have to pay from $1 to $1.50 or even a higher rate per thousand plants. This makes planting more expensive by increasing the cost per unit of production. Another disadvantage which may occur is the introduction of "black shank," carried into his field on poorly selected seedlings from infested beds.

SYMPTOMATOLOGY

The morphologic symptoms of tobacco damping-off appear in various manifestations depending on the age of the plants. Very young seedlings take on a dirty green color and die. The seedlings are so violently attacked that usually the time between the first symptoms and death is very short. On larger plants, which are not overcrowded and which have not developed a slender stem, if infected at the surface of the soil, the first symptoms consist of a wilting of the leaves. This is followed by a rapid blackening of the stem all the way up to the terminal bud. The stem tissues appear to shrink. These symptoms are usually produced by *Phytophthora Parasitica,* but may also be caused by *Pythium debaryanum.** When plants are too crowded, their stems are rather long. Here the symptoms may be evident either as small, lens-shaped or elongated lesions which do not extend far up the stem or as large lesions in the cortical tissues, originating near the soil surface and spreading up and around the stem, in a good many cases girdling it completely. These lesions are usually pale and are typical of the disease caused by *Pythium debaryanum.* Such plants will generally have the lignified tissues unaffected and when transplanted into the field will send out roots above the lesions and usually develop into normal plants. *Phytophthora Parasitica* produces very similar symptoms (see plates XXVII, XXVIII and XXIX.) Occasionally lens-shaped lesions will be formed on the stems and adventitious roots develop. When transplanted into the field during a rainy period such plants rapidly succumb to the attacks of the invading fungus. The organism in the small lesion commences activity on reaching the new environment. Such a con-

* Since these two pathogenes produce damping-off effects almost identical on tobacco they are here discussed as producing one disease.

dition had been thought to be due to a cause different from that of the damping-off but it has been found that *P. Parasitica* is the responsible agent. Roots of young seedlings are not usually affected as the pathogenes seem to spread more readily over the surface of the soil and infect the stems at soil level. However, roots of the larger seedlings may furnish the point of invasion. This is especially true in the case of *Phytophthora Parasitica.*

The leaves are also a common point of entrance for the pathogenes. The symptoms here consist of circular to irregular light-brown spots with light centers. The plesionecrotic zone between the unaffected and necrotic tissues is pale green. The spots rapidly increase in size and involve the entire leaf blade, and in later stages extend to the stem where blackening and death of the tissues occur and ultimately the death of the plant.

The histologic symptoms consist, in the first place, of hydrosis of the tissues, especially when infection occurs in the leaves. The cells of the invaded tissues are soon killed by toxins or related products secreted by the penetrating hyphae, and the cell walls and cell contents are blackened presumably as a result of the reactions with the fungous secretions. Collapse of the cells is followed by the dissolution of the primary walls of the mesophyll cells of the leaf or of the cortex of the root, as the case may be. Withering of the affected individuals soon follows. The vascular tissues, especially in infections by *Phytophthora Parasitica* are also stained and killed. This results in the wilting of the above-ground parts.

Signs

Sporangia are produced abundantly in the soil by *Phytophthora Parasitica* and to a lesser extent by *Pythium debaryanum.* However, *Pythium debaryanum* forms oospores in the soil and in the tissues of the affected organs. Oospores of *Phytophthora Parasitica* have not been found in the tissues of diseased seedlings. Chlamydospores are formed in the infected parts when either of the two pathogenes is present.

Etiology

The pathogene *Pythium debaryanum* was discovered and described by Hesse in 1874. It was named in honor of the famous German mycologist, Anton de Bary. There is now a long list of publications on this fungus, in which it is recorded on a large number of suscepts.

The other pathogene, *Phytophthora Parasitica* var. *nicotianae* was first described as *P. nicotianae* by Breda de Haan in 1896 (5). This

fungus was first associated by its discoverer, with the cause of the "lanas" disease; and known in America as the black-shank. A serious seed-bed trouble was found to be caused by the same fungus in the Deli district of Sumatra and at Buitenzorg in Java.

Tisdale (27) first reported the black-shank organism from America in 1922 as causing a typical damping-off in young tobacco plants. Later, in 1923, he (28) gave *Phytophthora nicotianae* Breda de Haan as the cause of black-shank.

In Puerto Rico damping-off of tobacco is due to *Pythium debaryanum* and *Phytophthora Parasitica* var. *nicotianae*. Rhizoctonia has not been found in the tobacco seed-beds, but an examination of some plants received from the Cayey region in January, 1930, showed the presence of this fungus, and it might well be that the pathogene is spreading in that section. *Sclerotium Rolfsii* was isolated from yellowed seedlings but it does not seem to be able to cause much damage.

That the Phytophthora on tobacco in Puerto Rico is *P. nicotianae* was established in a previous paper by the author (23). The cultures used in this investigation were the same isolates studied in connection with the black-shank disease. Ashby (1) in 1928 proposed the name *Phytophthora Parasitica* Dastur for a combination of species among which is *P. nicotianae*. Obviously he ignored any suscept relationship. Tucker (29) who has been unable to separate *nicotianae* from *Parasitica* on a morphological basis, regarded the difference in pathogenicity between the two as not of specific significance and, therefore, designated the fungus *P. Parasitica* Dastur, var. *nicotianae* Tucker.

The omnivorous *Pythium* in Puerto Rico has been isolated from many suscepts. The isolates when compared with known cultures of *Pythium debaryanum* have shown only slight or no morphological differences.

Pathogenicity

The pathogenicity of our isolates of *Pythium debaryanum* and *Phytophthora Parasitica* was established by a series of experiments.

The following list of isolates used is given with their sources;

P 1. *Phytophthora Parasitica*, from a tobacco seed-bed in Cayey;

P 2. *Pythium debaryanum*, from a tobacco seed-bed in Caguas;

P 3. *Phytophthora Parasitica*, from a tobacco seed-bed in Cayey;

P 4 to P 14. *Phytophthora Parasitica*, tobacco black-shank Caguas;

P 16. *P. debaryanum*, tobacco plant with black-shank lesions, Cayey;

P 17. *P. Parasitica,* disease of young transplants; Cayey;

P 18. *Phytophthora* sp., tomato damping-off or bending-over, Río Piedras;

P 19. *Phytophthora* sp., eggplant fruit rot;

P 20. *Pythium debaryanum,* cucumber damping-off, Río Piedras;

P 216. *Phytophthora Parasitica,* obtained from Trucker who obtained it from Tisdale—the Florida black-shank fungus;

P 217. *Phytophthora* sp., obtained from Tucker but source unknown.

Not all of these isolates were used in all experiments because they were gradually accumulated during the course of the investigations.

The flats in which most of the pathogenicity experiments were conducted were prepared as follows: A wooden box 2 ft. × 2 ft. was filled with good compost. A frame about 2 ft. high was built around each box. Cell-o-glass was then fastened tightly to this frame, the lower edge extending about 2 in. below the upper edge of the sides of the box. This insured the contents against possible infestation from external sources. Frame-covers were also provided in order to prevent rain water from falling into the box.

As soon as the flats were filled with the soil compost, they were sterilized with a 1–50 formaldehyde solution, at the rate of 1 gal. per sq. ft. or 4 gal. to each flat. After the soil was well soaked with the solution it was covered with burlap for 48 hours.

A week after disinfestation, the soil was infested with cultures of the isolates to be tested. At the end of 7 days after infestation of the soil seed was sowed of the types "Borinquen" or Connecticut Round Tip (very susceptible to black-shank) and a native type called "País" (resistant to black-shank). The beds were heavily watered with boiled water every morning, until the experiment was completed.

Expt. 1: Twenty-four flats were used, three were left uninfested to be used as checks while the remaining 21 were infested on Dec. 22, 1926 as follows: A set of 3 flats with P 1, P 2, P 3, P 4, P 10, P 13, and P 14. These flats were sown on Dec. 29 as follows: 2 flats in each set with "Borinquen" seed the remaining flat with "País" seed. The check was similarly seeded.

The results observed at intervals of 2 days until Jan. 21, 1927 were as follows:

(*a*) There was an even germination on all the 24 beds.

(*b*) P 1 (*Phytophthora Parasitica*) produced a virulent infection on Jan. 10 and in 3 to 4 days had destroyed all the seedlings in the three flats alike.

(*c*) P 2 (*Pythium debaryanum*) showed infection on Jan. 10, and by Jan. 18th all the seedling in the three flats had been killed.

(*d*) P 3. The first symptoms were observed on Jan. 10. Later on Jan. 13 there were only a few plants standing.

(*e*) P 4 and P 10 showed the symptoms later than in the preceding three cases but by Jan. 21 nearly all the plants had been destroyed.

(*f*) P 13 and P 14 showed infection on Jan. 18 and by Jan. 21 there were only a few seedlings unaffected.

(*g*) No damping-off developed in the checks, the plantlets attaining a normal development.

There are two conclusions to be drawn from this experiment, namely, that both *Pythium debaryanum* and *Phytophthora Parasitica* cause severe damping-off of tobacco seedlings and that there is no difference in the resistance or susceptibility of the two varieties of tobacco used.

A second experiment was conducted in which infestation was made 16 days after germination and when seedlings were making a vigorous growth. Ten-day-old mass cultures of P 216, P 217, P 16, P 17, P 18, and P 20 on cotton-seed meal agar were employed.* The cultures were broken up into small fragments, poured into tin pans and water was added so as to obtain a good dilution. This was then sprinkled over the seedlings and washed into the soil with water from sprinkling cans.

A week after infestation symptoms of the disease had appeared in all of the infested flats except those with P 18 and P 217. Spots on leaves appeared only in the flats infested with P 17 and P 216.

From these results it may be concluded that, (1) the Florida black-shank pathogene *P. Parasitica* (P 216) causes typical damping-off; (2) the *Phytophthora* sp. (P 217) is non-pathogenic to tobacco seedlings; (3) two *Pythium* strains from tobacco (P 16) and cucumber (P 20) produce typical damping-off; *P. Parasitica* (P 17) from transplant tobacco causes typical damping-off, and (5) a *Phytophthora* sp. which causes bending-off in tomatoes does not cause the disease on tobacco.

* Prepared by adding to 40 grams of cotton-seed meal, 4 grams of agar-agar and water enough to make the volume to 200 cc. in an Erlenmeyer flask, then heating in the autoclave at the usual temperature for sterilization.

Life History

An account of the life history of *Phytophthora Parasitica* has been given in another paper (23). In the account of the life history for *Pythium debaryanum* given below, it will be seen that it is similar to that of the former. The primary cycles are initiated on the seedlings by inoculum from the soil or from bits of diseased plants carried into a pathogene free seed-bed by agents of dissemination. The inoculum consists of mycelium from infested debris and of oospores, chlamydospores, conidia, and zoospores. Inoculation is effected by the growth of the inoculum to the susceptible part or by its transportation to the suscepts by agents of various kinds. Surface water currents, laborers, animals, and probably burrowing insects as well as other agents serve to disseminate it.

With favorable conditions of temperature and moisture, the oospores, and probably the chlamydospores may be assumed to germinate. Laboratory attempts to germinate the oospores and chlamydospores have been unsuccessful, an experience which confirms that of other writers. The germ tubes from the oospores must come into contact with the suscepts, or, failing to do so, they develop into a mycelium in the soil.

The mycelium under adequate temperature and humidity conditions assumes a very active role. Zoosporangia and conidia are developed at the end of special hyphae. The zoosporangia germinate in the water on the suscepts' surfaces or in pools, giving rise to zoospores. These and the conidia germinate by sending out a germ tube. The term conidium is used here in the sense of many authors for the sporangia which do not germinate by zoospores but by a germ tube. There seem to be no grounds for regarding these as distinct structures, both are, undoubtedly, genetically alike and the manner of germination may be, after all, influenced by external factors not yet well understood.

Germ tubes and the hyphal tips of the mycelium coming in contact with the susceptible surfaces penetrate the cell-walls of the tissues. Hawkins and Harvey (15) present evidence to show that the hyphae of *Pythium debaryanum* penetrate the cell-walls of the potato tuber by means of mechanical pressure. The germ tubes or hyphae may enter the plant through the stomata. Zoospores of *P. Parasitica* occasionally brought into contact with the leaf surface at the margin of the blade by splashing rain drops or other means, send their germ tubes into the tissues, in this manner giving rise to the characteristic spots. We have been unable to get infection of leaves with zoospores of *Pythium debaryanum.*

Once the hyphae or germ tubes find themselves inside the suscept tissues they begin to invade these. The mycelium becomes much branched and advances in all directions. The hyphal branches penetrate the cell-walls or may penetrate in an intercellular manner. The hyphae of *Pythium debaryanum* become constricted at the place where they pass through a cell-wall. However, this phenomenon has not been observed in *Phytophthora Parasitica*. It has been generally accepted that *Pythium debaryanum* secretes a substance which kills the cells of the suscepts in which it occurs.

During the course of the development of the pathogene on the tender stems and leaves of the tobacco seedlings zoosporangia and conidia are produced. These are more abundantly produced by *Phytophthora Parasitica* but on the other hand, *Pythium debaryanum* produces an abundance of oospores in the tissues and in the surrounding soil; while the former produces only a few. Chlamydospores are produced by both pathogenes. The sporangia germinate readily under favorable environmental conditions and their zoospores together with the germinating conidia (probably also chlamydospores and oospores) furnish the inoculum for the secondary cycles. The primary cycles end with the death of the seedlings. Only in a few cases, when affected plantlets have developed vigorously, does recovery from the disease occur, and then only when the pathogene involved is *Pythium debaryanum*. Seedlings at any age or period of development, when infected by *Phytophthora Parasitica* die. After the destruction of the plants in a bed or part of them, the land is usually left idle. The pathogenes now enter into a new phase of their life history. They are able to hibernate in the debris of the dead seedlings or in organic matter of any kind. Here they spend a saprophytic life awaiting the reappearance of any one of their suscepts.

Epiphytology

Environmental conditions seem to affect both pathogenes similarly. The amount of moisture in the soil seems to be the most important factor determining the spread and severity of these pathogenes. Under conditions of extreme humidity the disease plays havoc with seedlings. It is natural to expect this with pathogenes which have such life habits and characteristics. In sections where rainfall is slight there is seldom any fear of damping-off. Temperature variation is not an important factor in Puerto Rico since favorable temperatures prevail through the year. In our experiments, conducted at all seasons, temperature fluctuations have not appeared to influ-

ence the occurrence and spread of damping-off very much. Seed-beds shaded with cloth are usually more liable to suffer severely. In those where no shade has been used, the disease has been checked with less difficulty. That some beds of seedlings have been saved by removing the cloth and allowing the sun rays to penetrate directly to the surface, indicates that the reduction of the moisture on the surface of the soil and on the plants, removes the chances of spread and development. The elevation at which the seed-bed is located in Puerto Rico seems to have little influence on damping-off.

Damping-off is usually severe in beds which have been heavily fertilized with nitrogenous fertilizers. This is true of soils manured with the more soluble salts such as nitrate of soda, and beds periodically watered with solutions of the salt. But organic manures seem to afford the best conditions for its development. Fresh barnyard manure furnishes a good medium for the pathogenes already existing in the soil. Barnyard manure in Puerto Rico is worse than nothing in the preparation of seed-beds and its use should be discouraged. Fortunately, the average grower has learned this from experience and there is little likelihood of its being used to any great extent. However, when seed-beds are properly disinfested and well managed there will be little danger from organic matures.

Thick sowings are a constant danger in Puerto Rico. In spite of the fact that during the last two decades the growers have been advised by private parties or by government agents to make as light sowings as possible, the present day growers still insist in getting a very large number of plants per unit area. The experiences of the season of 1927 show this very plainly. We found cases of growers who had been managing tobacco seed-beds for the last ten or twelve years for a certain tobacco company, and had then used only about 2 pounds of seed to the acre. When they started in work of their own they made sowings at the rate of 4 pounds to the acre. The result was an overcrowding of seedlings in the seed-beds. With the coming of the rainy season, damping-off occurred and the destruction was almost complete. Experience in Puerto Rico shows that not more than 1.5 to 2 pounds of seed should be sown to an acre.

CONTROL

ERADICATION

General Considerations and Miscellaneous Practices

The control of tobacco damping-off is at present one of the problems which the tobacco growers have to face. Practical treatment

for beds before or after the germination of the seed will be welcomed by the growers of tobacco in Puerto Rico.

The first known and most general treatment of the land for the control of plant diseases ever recorded for Puerto Rico is the destruction of the causal organism by fire. The first records of the use of this means for the cleaning or sanitation of soils intended for seed-beds are lost in the rather obscure history of Puerto Rican agriculture. The pioneer tobacco growers in the island did not know that plants would die from disease; when tobacco culture was first attempted their virgin lands probably were free from infestation. In some sections of the country the clearing of the land was followed by burning of the trash, shrubs, and trees. In other cases (especially recent ones, which the writer has seen), the beds were prepared in places where the plant "maya" (*Bromelia Pinguin*) had been growing for years. This large terrestrial bromeliaceous plant grows thickly in waste places and along fences and these seemed favorite places for tobacco beds. When set on fire these plants burn slowly since their leaves are more or less juicy. This slow burning develops an intense heat, which is maintained in the soil for a relatively long time. This may be an explanation for the small amount of damage produced by the so-called rot or damping-off in seed-beds prepared in such sites. Without knowing it these growers with their rude methods were preventing a serious trouble. Later as seed-beds had to be increased in extent the patches of "maya"-covered land had to be abandoned for other waste lands. In other sections this system was unknown, but instead grass was burned over the soil. Gradually with the clearing of land the amount of material to be burned on the soils intended for seed-beds decreased and the seed-beds received no treatment whatsoever. The disease called here "pudrición" (rot) or "salcocho" (damping-off) began to increase year after year until it is now widespread over the entire island. The method of firing as used by the first growers was an effective one under the conditions of slight infestation then prevailing. Its effectiveness decreased when beds were less frequently exposed to the action of the heat and when they received contamination from adjoining fields through surface drainage.

A second eradicatory measure, but a poor one, used by our growers has been the removal of the diseased seedlings from the fields as soon as the disease appears. This practice is effective in the control of some diseases and, most certainly, in those cases where the disease occurs in isolated individuals; but in the case of tobacco damping-off, especially in Puerto Rico, it is very dangerous. The

type of labor employed in this kind of work does not commend its use. A laborer who removes diseased seedlings will only serve to spread the inoculum over healthy beds. In the majority of instances the rotting plants have been scooped out and thrown into the ditches where they await the coming rains to wash them down to other ditches and into other beds. After seedlings have been removed it is the practice to dust the bare spots with slaked lime. This lime does not kill the fungus and observations show that even indirectly it will not check the spreak of the pathogenes. It is an unfortunate thing that methods like this should even be mentioned in the literature as effective. Bunker (4) says "The practice of scraping off the infected soil and applying lime is good." However, immediately after, he describes a method which is more satisfactory and which shows there was no need of approving the former. This latter method consists in spraying the infested areas well into the surrounding heathy plants with a 1–25 formaldehyde solution. After soaking with this solution the diseased plants may be removed from the beds. We are of the opinion that this is a good measure to follow. We have used a slightly lower concentration, a 1–30 solution, with equally good results. Once the infested spots have been thus drenched, the removal of the infected plants seems to us rather unnecessary. There is little likelihood that these will serve as sources of inocula. Recently we have succeeded in obtaining excellent results with a new copper preparation—a copper fluosilicate dust. This material has a very high content of copper in the soluble form. When applied to infested spots in tobacco beds it soon burns the foliage and stems. At the rate of about 8 grams to the square foot of surface it gives as good results as the 1–25 or 1–30 formaldehyde solution. It has the advantage over the latter in not having that penetrating, undesirable odor. Further, its action is longer continued than that of formaldehyde. It is a good plan to sprinkle the beds with water after dusting with copper fluosilicate dust. A 1–200 Uspulun solution at the rate of 1 gallon to the square foot of surface has also given satisfactory results but none of the treatments are as simple and effective as dusting with copper fluosilicate.[1]

Of historical interest is the case reported by Loew (20) of a certain Mr. DuBois who had introduced a new system for preventing the disease, by transplanting the young seedlings into a second seedbed before they were set in the field. It is of further interest to note that the writer observed exactly the same system employed by

[1] After this paper had been prepared the writer was informed that this compound will not be prepared any longer.

the farmers of Colombia, South America, in 1929. This is a rather primitive method which is inapplicable and uneconomical on a large scale.

Eradication of pathogenes by soil treatments before sowing the seed

Steaming of the soil

It is highly improbable that soil disinfestation by means of steam can be adapted to Puerto Rican conditions. Its successful use in other countries makes it a very satisfactory treatment under certain conditions. Atkinson (2) in 1895 suggested the use of steam for the control of damping-off in plant beds in severe cases. Gilbert (14) finds steam sterilization to be the best means of preventing tobacco root-rot. Clinton (7) comparing steam sterilization with the formalin drench in the control of tobacco root-rot found the steam treatment of beds as effective, with the additional advantage of being more efficient in killing weed seeds. Johnson in 1914 (19) in studies on the control of damping-off in plant beds with special reference to tobacco damping-off (*Pythium debaryanum* or *Rhizoctonia*) compared steam disinfestation with other means of treating beds, and concluded that the former was the most satisfactory method of preventing the disease.

In our opinion Puerto Rican growers will not resort to this means of eradication of the damping-off pathogenes. The economic conditions in the tobacco regions are more and more pressing every year and expense is the most important item. The cost of machinery and equipment needed in the treatment of plant beds by the steaming method is so high that it is prohibitive even for the more wealthy growers. It might be possible to introduce it by cooperative undertaking; but it is improbable that this will be accomplished.

The formaldehyde drench

History

It is generally admitted that the treatment of soils with a formaldehyde solution is an effective method in the control of fungus seedbed troubles. This disinfestant was first employed in eradicating soil fungi by Selby (24), in 1889 and 1900. He applied it to soils infested with the onion smut fungus *Urocystis cepulae* Frost. Later in 1906, this same investigator (25) recommended the treatment for the control of Rhizoctonia bed-rot and Thielavia root-rot on tobacco seedlings. It consisted of applications of a formaldehyde solution

(2½ pounds of 40 per cent fomaldehyde in 50 gallons of water) at the rate of 1 gallon per square foot of bed surface. The concentration is much weaker than that now recommended. This appears to have been the first attempt to control tobacco seed-bed troubles by the use of formaldehyde. In 1907 Clinton (l. c.) obtained good results with formaldehyde in the treatment of plots and crocks for the control of the Thielavia root-rot of tobacco. He used a 1–100 solution and applied it at the rate of 1 gallon to the square foot. Clinton says, "We believe that the formalin treatment is a very efficient and convenient method of protecting tobacco beds against root-rot and possibly the damping-off trouble." Yet, Selby (l. c.) had found the damping-off fungus (*Pythium*) occurring in treated seed-beds.

In 1909 Gilbert (l. c.) reported the results of beds treated with 1–200 and 1–300 solutions at the rate of ¾ gallon per square foot for the control of tobacco root-rot as only a little better than the untreated. In the same publication he states that a 1–100 solution might be used advantageously. In 1914 Johnson (l. c.) reported effective control of damping-off (*Pythium debaryanum* or *Rhizoctonia*) of tobacco with 1–50 formaldehyde solution applied at the rate of 2 quarts per square foot of surface. This is the concentration and rate recommended today.

Many other papers have appeared recommending formaldehyde for the eradication of damping-off. Among these there is one by Chapman (6) for tobacco damping-off.

Although the formaldehyde disinfestation of tobacco seed-bed soils in Puerto Rico seems prohibitive on account of the cost, experiments have been made to determine the efficiency of the treatment as practiced in other countries in the control of damping-off.

Experiments in Puerto Rico

(1) A set of trials was started in December 1926 as follows: twelve flats 2 ft. × 2 ft. and one foot deep were filled with a soil mixture containing about 50 per cent barnyard manure. Ten of these flats were drenched with a 1–50 formaldehyde solution, at the rate of two gallons per flat or ½ gallon per square foot of soil surface. Two flats remained untreated and kept about 10 yards away from the treated flats. The treated flats were covered with burlap for about 2 days. Tobacco seed was sown in the twelve flats a week after treatment. The notes taken a month after germination were as

follows: complete damping-off in the two untreated flats, some damping-off in four of the treated flats; six of the treated flats were healthy. (2) This experiment was repeated in flats where the chances of contamination after treatment were obviated. They were raised about 2 feet from the ground on a wooden platform. The flats were surrounded by frames of cell-o-glass (impermeable screen) about two feet high and nailed tightly to the sides of the boxes. Another frame was employed as a cover. The soil used was the same as for the first experiment. Six such flats were treated with the 1–50 formaldehyde solution, one was kept as a check. Damping-off occurred virulently in the check flat and in two of the treated flats. (3) In a third case data was obtained on 10 flats totalling about 90 square feet of surface which had been treated with a 1–50 formaldehyde solution at the rate of 1 gallon per square foot and planted with "Virginia Blanco" tobacco seed. Damping-off appeared in the flats about two weeks after germination of the seed. Reinfestation of the soil may have occurred, although this is not very probable. (4) In a fourth experiment 8 flats (5 ft. × 3 ft.) were disinfested as above in October 1927. The flats were covered with cell-o-glass movable frames soon after disinfestation. The flats were watered liberally, twice a day. Final observations on November 26, 1928 showed no damping-off in any of the flats. (5) A fifth experiment was started in the latter part of November and completed in December of 1927, using cultures of *Phytophthora Parasitica* and *Pythium debaryanum* for infesting the soil which contained in itself a high percentage of barnyard manure. The cultures were about one week old, vigorously growing in oatmeal agar. Each flat received 500 cc. of the culture. Six flats 5 ft. × 3 ft., were used, two for each one of the pathogenes and the remaining two as uninfested checks. The four infested flats and one of the checks received an application of 1–50 formaldehyde solution at the rate of one gallon per square foot of soil surface. This was made two weeks after infesting the soil. It was found in this trial that the *Pythium debaryanum* flats and the treated check flats were healthy at the end of the experiment; the *Phytophthora Parasitica* flats developed small areas of the disease; while the untreated checks developed damping-off which soon killed all the seedlings. (6) In a sixth case observations were made and notes taken on a one-acre tobacco plant bed disinfested by the Porto Rican Leaf Tobacco Co. with a 1–50 formaldehyde solution at the usual rate. The treatment was made in

December of 1927 soon after clearing part of a field which had been growing a tobacco seed-bed sown early in the fall. When the last notes were taken on January 20, 1928, damping-off had appeared in some of the treated beds. This occurred after a rainy period. Generally the treatment was effective. It may be noted that the application was not as thorough as is recommended, when one considers that most of the ditch space was left untreated. Then also the spread from one ditch to the other by the feet and shoes of the laborers who attend to the watering and weeding is an important factor not to be overlooked. The weather was, however, especially unfavorable for the development of the disease; rains were light and there was maximum sunlight. Therefore, the rather unsuccessful results in this trial must not be attributed to the treatment alone but the other factors influencing the severity of the disease must be considered.

Summarizing our own experiments and experiences with the formaldehyde treatment, it is concluded that: (1) a 1–50 concentration applied at the rate of ½ gallon to the square foot of surface is not an absolute disinfestant for soils infested with *Pythium debaryanum* or *Phytophthora Parasitica*, at least under Porto Rican conditions. (2) Formaldehyde at a 1–50 dilution and applied at the rate of 1 gallon per square foot is probably ineffective in eradicating the pathogenes. (3) *Phytophthora Parasitica* appears to be slightly less susceptible to the sterilizing action of formaldehyde than *Pythium debaryanum*. (4) The results of treatments are always shifted one way or the other by environmental conditions. (5) The location of plant beds in the island is such that reinfestation is very apt to occur at the time of the heavy rains. Infested soil may be washed down the hills into the beds. Under such circumstances the disease will develop and spread rapidly in the formaldehyde-treated soil. (6) The method is too expensive and, therefore, inapplicable in Puerto Rico.

Other Chemical Treatments

History

On tobacco beds Johnson (l. c.) reported benefit from the application of 0.4 per cent and 0.5 per cent sulphuric acid by weight. This amount he held to be as high as could be used in order to permit the germination of the seed. It appears that the little work done with sulfuric acid on tobacco damping-off has been unsuccessful.

In 1907 Horne (17) recommended the use of Bordeaux mixture for combating the damping-off of tobacco seedlings. Later in 1908

he (18) gave the treatment and preparation of the mixture in more detail. He used 6 lbs. of copper sulphate and 15 lbs. of slaked lime paste in 50 gallons of water, applying 1 gallon of the mixture to every 10 square feet of soil surface. This seems to be the first attempt at a practical solution of the problem of tobacco seed-bed troubles with chemicals other than formaldehyde.

In 1914 Johnson (l. c.), in experiments on the control of tobacco and garden cress (*Lepidium sativum*) damping-off, found inhibitory action on germination of seedlings with lime-sulfur, potassium sulphide, copper sulphate and mercuric chloride treatments. For this reason he thought there was little value in those disinfestants as soil fungicides. In the same experiments he obtained a decided decrease of the disease in soil treatments with Bordeaux mixture.

In 1919 d'Angremond (10) stated that tobacco could be protected in the nurseries with Bordeaux spraying and, later in that year, he (11) gave results of treatments of dessa manure or native compost for the destruction of *Phytophthora Parasitica*. He used benzine at the rate of 2.5 liters per cubic meter with no favorable effects; but his application of 510 grams of carbon bisulphide to the cubic meter was quite successful. This same worker (12) reported in 1920 the treatment of manure with copper sulphate as inadequate but carbon bisulphide was again successful. The latter treatment is, however, not practicable.

In 1925, Cook (8) recommended the use of 5–5–50 Bordeaux mixture, applied every 3 or 4 days for the control of tobacco damping-off. No information on the quantity of spray per unit area was given.

Major (22) found mercury compounds were injurious to tobacco plants in seed-beds when he used it for the control of the Thielavia root-rot.

In 1926, Bunker (3 and 4) advocated the Bordeaux mixture treatment but applied every 2 or 3 days. He did not give the amounts of the mixture to apply.

Thomas (26) controlled damping-off of tomatoes caused by *Phytophthora* sp. with copper carbonate, mercuric chloride and Uspulun. Mercury compounds were effective against Rhizoctonia in cabbage and tomatoes; copper carbonate and two forms of colloidal copper were ineffective in controlling this disease.

Lucca (21) has more recently reported control of tobacco damping-off with treatments of a 5–8–50 Bordeaux mixture. He says spray-

ings were made every 3 or 4 days but fails to give the amount of the spray per unit area.

More recently Doran (13) has found that he can protect tobacco against black root-rot and damping-off with an application of 1 to 1.2 per cent acetic acid solution at the rate of one-half gallon per square foot.

It is evident from the foregoing review of most of the investigations and reports on treatments for tobacco damping-off that formaldehyde and acetic acid are, in a general sense, effective treatments against damping-off of tobacco, but the objection raised against them is their inefficiency in protecting seed-bed soil from reinfestations that usually follow. To be applicable, cost excepted, to conditions in our country, a system, of more or less permanent seed-beds should be installed whereby the soil, once disinfested, could be protected from reinfestations. As previously stated, the main disadvantage would be in the cost of the treatments. The Puerto Rican growers are in need of cheaper methods of control. The use of mercury compounds has been discouraging on tobacco seedlings, injury having been reported. Bordeaux mixture has been used to advantage in Cuba and in Puerto Rico, but the details of the treatment have not been worked out on the basis of its usefulness, practicability and economy. Recently some new copper dusts have been tried by Thomas (l. c.) against the damping-off of tomatoes and other vegetables with such beneficial effects that it appeared to us that they might be promising in the control of our tobacco seed-bed troubles. These have the very desirable quality of being applied in the dust form, doing away with the inconveniences of spraying. This virtue is to be courted in any disinfesting treatment for soils.

A number of copper and mercury compounds have been used in our experiments with formaldehyde as a check. Mercuric chloride and two organic mercury compounds whose commercial use is probably prohibitive, have been used for the sake of comparison. Acetic acid has also been employed in the experiments.

In the following experiments, unless otherwise stated, the flats used were about ten inches deep. They all were protected from unnecessary insolation by cheesecloth shade. Ample drainage was afforded by means of ditches between the beds and flats. In every case much care was exercised to prevent the spread of the pathogenes from one bed or flat to the other. The seed was mixed with cottonseed meal to facilitate even distribution.

Experiments with Corona Copper Carbonate

The copper carbonate dust employed in the following experiments contains about 20 per cent metallic copper.

Experiments in flats and experimental beds

In all experiments, unless otherwise specified, the soil was a compost made of six parts of heavy loam, one of well rotted barnyard manure and one part of sand.

Rate of application. Five sets of four flats each, 5 ft. × 6 ft. × 1 ft. were infested with isolates P 1, P 2, P 3, and P 10 and P 13, while a fifth set of four flats was left uninfested to serve as checks. An application of ½ gram, 1 gram, 2 grams, and 4 grams per square foot, respectively, of the chemical was made in each set of infested flats. It was dusted over the surface uniformly with a fine-mesh sieve and then well raked into the upper inch of soil. The soil was kept moist in all the flats. The twenty-five flats were sown thickly with Connecticut Round Tip seed two weeks after treatment. All the flats were kept well watered during the course of the experiment. Observations were made at short intervals.

Two weeks after germination, it was found that damping-off was quite severe in nearly all the flats. A second application was made to all flats (except the checks) at the same rate as before.

The dust from the second application was well washed into the soil by sprinkling the plants with water. The results are given in Table 1.

At the end of the experiment it was evident that: (*a*) all seedlings in the checks were affected; (*b*) the majority of plants died in the flats infested with P 1 and P 3 and treated with 2 applications of ½ gram, 1 gram, and 2 grams per square foot of surface; (*c*) the majority of the plants died in flats infested with P 2 and P 10 and P 13 when applications of ½ gram and 1 gram were made; (*d*) a fair control of the disease in flats with P 1 and P 3 when 4-gram applications were made and in those with P 2 and P 10 and P 13 when either 2- or 4-gram applications were made.

In another experiment with Corona copper carbonate, the dust was applied to the beds (20 ft. × 3 ft.) at the rate of four grams to the square foot of surface. The first application was made one week before the seed was sown and a second application followed two weeks after germination of the seed. In this experiment three beds were infested with a mixture of cultures of *P. debaryanum* and *P. Parasitica;* and of these beds one was left as a check. At the time when seedlings were of transplanting age no disease was ob-

served in the treated beds, while the seedlings in the check bed had been mostly destroyed. (See plates XXX, XXXI and XXXII.

TABLE 1

EFFECT OF CORONA COPPER CARBONATE. ONE APPLICATION A WEEK BEFORE SOWING OF SEED, A SECOND 2 WEEKS AFTER GERMINATION

Application per sq. ft.	Results—Severity of Disease							
	P-1		P-2		P-3		P-10 & P-13	
	March 21	April 11	March 21	April 11	March 21	April 11	March 21	April 11
½ gram.....	2 diseased areas	90%*	1 diseased area	95%	2 diseased areas	90%	3 diseased areas	90%
1 gram.....	3 diseased areas	90%	2 diseased areas	90%	2 diseased areas	90%	3 diseased areas	90%
2 grams.....	2 diseased areas	90%	1 diseased area	40%	1 diseased area	80%	1 diseased area	30%
4 grams.....	3 diseased areas	30%	3 diseased areas	10%	1 large diseased area	10%	2 diseased areas	10%
Check.....	4 diseased areas	100%	1 diseased area	100%	5 diseased areas	100%	4 diseased areas	100%

*The percentages given are for estimates of the area of seed-bed destroyed.

Judging from the results of the above experiments it would seem that two applications of 4 grams of copper carbonate, one before sowing the seed, and the other two weeks after germination might control the disease. It appears also that even 2-gram applications will be sufficient to control *P. debaryanum* (P 2).

Time of application and effect on disease control. In order to test the effect of Corona copper carbonate when applied at various times before and after seed sowing, eighteen flats, 7.5 ft. × 3 ft., were infested with *Phytophthora Parasitica* prior to treatment and seeding.

One set of three flats was selected as check and the remaining five sets of three flats each were treated in the following order: set No. 1 three weeks before; set No. 2 two weeks before; set No. 3, one week before; set No. 4 at time of sowing; and set No. 5 one week after sowing.

At the end of three weeks after germination the disease had appeared in all the flats and, therefore, a second application of four grams of the dust was made on all flats except the checks. The disease continued with great severity. The final observations were made two weeks after the second application and seedlings which still remained apparently healthy were removed and examined for lesions. Only those seedlings showing no lesions were classed as healthy. The percentage of healthy seedlings in the stand at the close of the experiment was as follows: set No. 1: 14.98, set No. 2: 12.44, set No. 3: 5.35, set No. 4: 11.09, set No. 5: 20.06, and

check 2.09. It should be noted that these figures do not represent the percentage of the original population. The figures of healthy seedlings on the basis of the latter would have been extraordinarily small.

The results show that there was no effect of time of application of dust on control. An only slightly but not significant favorable effect occurred when the dust was applied three weeks before the time of seed sowing. From these results it would seem that two applications of 4 grams of copper carbonate are not effective in the control of damping-off, a conclusion which is not in harmony with previous results.

Relations between amount of water and effect of treatment. With the purpose of determining the relation between different amounts of water and the effect of copper carbonate on seedlings, twelve flats (5 ft. × 3 ft.), protected from the rain by cell-o-glass frames, were treated with 3 grams of the dust and watered as follows:

Flats Nos. 1 & 5—1 gallon of water. Morning.
Flats Nos. 2 & 6—1½ gallons of water. Morning.
Flats Nos. 3 & 7—2 gallons of water. Morning.
Flats Nos. 4 & 8—2½ gallons of water. Morning.
Flat No. 9—1 gallon of water. Morning and afternoon.
Flats No. 10.—1½ gallons of water. Morning and afternoon.
Flat No. 11—2 gallons of water. Morning and afternoon.
Flat No. 12—2½ gallons of water. Morning and afternoon.

The quantities of water given are in each case for the entire flat, i. e., for 15 square feet of surface. It may be noted that the amount of water in the soil at the beginning of the experiment was not determined, but the soil was fairly moist.

The results may be summarized as follows:

(*a*) Better development of seedlings in flats 9 to 12 where two waterings were made than in the flats 1–8 (one watering).

(*b*) Flat No. 9 with only one gallon of water for each application, resulted in less germination and the seedlings made less progress than in any of flats 10, 11, or 12, but the seedlings were healthy.

(*c*) Flats 1 and 5 had very few plantlets, but they were healthy.

(*d*) Flat No. 11, two waterings every day, made a slightly better growth than flats 4 and 8 which received one watering of a little higher quantity.

(*e*) The last and most important—there was no injury from the copper carbonate in any of the flats.

Field Treatments

A series of treatments were made under field conditions making applications of the copper carbonate at the rate of four grams to the square foot of bed surface. These trials were conducted on various farms of the Cayey–Aibonito tobacco section. In none of these was the dust applied before seed sowing and always after the disease had made its appearance. The seed-beds chosen represent conditions of both high and low infection.

Conditions of light infection. Two seed-beds in Aibonito in which damping-off had appeared, were treated in October 1927 with the dust at the rate of four grams to the square foot. The disease was evident as small rotted areas here and there. One of the seed-beds had an area of a little more than 7,000 square feet; while the other was of about 4,200 square feet. These treated beds in each case were surrounded by other beds which received no treatment. When treatments were made the plants had reached the stage when leaves are the size of a half-dollar. The observations in both trials showed that the disease had not been checked and, therefore, a second application, at the same rate as the first was made two weeks later.

The results at the time when seedlings were transplanted showed no beneficial effects of the treatment. A general inspection of the series treated with copper carbonate and that without any treatment showed many diseased areas in the latter, where seedlings had been almost completely destroyed by the disease. On the treated beds these diseased areas were less numerous and, seemingly, smaller in extent. More definite results were obtained by making counts of seedlings removed from portions of the beds in the two series. Two contiguous beds, one treated and a check, were selected. Starting at the lower portion of the seed-bed and about ten feet from the end of the bed, a sector three and one-half feet wide by two feet in depth was marked off. At intervals of 15 feet three other sectors were measured in like manner. This was done for both beds, so that one sector in the treated bed corresponded to a sector in the check. The healthy seedlings existing in the beds at the end of the experiment were pulled and counted. The results were as follows:

Sector	Treated bed	Check	Difference
1	638 seedlings	306 seedlings	332 seedling
2	712 seedlings	508 seedlings	204 seedlings
3	617 seedlings	611 seedlings	6 seedlings
4	681 seedlings	708 seedlings	–27 seedlings

These differences were studied statistically by Fisher's method

for the determination of significance of differences of means, and odds of less than 2 to 8 were found, indicating very insignificant results. It must be concluded, then, that the treatment was ineffective. Objection may be raised to the value of these results and the conclusions therefrom, since the observations can be regarded as insufficient. Yet the odds obtained were so low that had a larger number of observations been made, the results would have been found to demonstrate ineffective treatment.

The efficiency of the treatment was, no doubt, influenced negatively by the extremely favorable conditions of atmospheric and soil moisture which prevailed, and by the large quantity of seed which had been sown (5 lbs. to the acre), which tend to favor the increase, spread, and severity of the disease. It may be added that some of the chemical may have been washed away by the rains.

Two other field trials of two four-gram applications were made in Cayey, one in the Model Farm and the other in a private farm. The treated portion of the first seed-bed was about 2,830 square feet. At the time of the first application the seedlings were about three weeks old and several areas of the disease had appeared in various places. The second application followed the first by ten days.

Counts were made in the first seed-bed because the disease did not spread from the original spots in the treated beds and it was thought that the general stand and freedom from lesions was sufficient to serve as a basis for conclusions on the effect of the treatment. The untreated beds suffered severely from the disease and no plants were used for planting. This experiment was considered a success.

In the second trial counts were made as described above except that sectors were marked off at intervals of 10 feet instead of fifteen feet, and eight observations instead of four were made. The results expressed as healthy seedlings were as follows:

Sector	Treated beds	Check	Difference
1	232 seedlings	238 seedlings	-6 seedlings
2	221 seedlings	307 seedlings	-86 seedlings
3	532 seedlings	281 seedlings	251 seedlings
4	813 seedlings	453 seedlings	360 seedlings
5	691 seedlings	503 seedlings	188 seedlings
6	447 seedlings	553 seedlings	-106 seedlings
7	559 seedlings	564 seedlings	- 5 seedlings
8	837 seedlings	684 seedlings	153 seedlings

These differences when studied statistically by Fisher's method were found to be insignificant, with odds of only slightly over 4:1.

The treatment, according to the results of two experiments, was

unsuccessful while a checking of the spread of the disease was obtained in one experiment. Whether the conditions of infestation were higher in the check beds of this experiment where control was secured was not determined at the time the experiment was begun, but the random selection of the beds is in favor of the view that this did not happen. It may be concluded that the evidence from three field trials with copper carbonate applied to beds where there is slight infestation of the disease agents, shows that the treatment is unsuccessful.

Conditions of heavy infection. A section of a tobacco seed-bed (about 6,000 square feet) on a hillside in Cayey, with a severe infection of damping-off (about 40 per cent of the bed destroyed) was dusted with copper carbonate at the rate of four grams per square foot of surface. The results were unsatisfactory, probably due to the fact that the treatment was made when the disease was well advanced. It should be added that heavy rains prevailed during the time before and after the application of the dust.

Effect of Copper Carbonate on damping-off of vegetables

In the autumn of 1927, twenty-eight six-foot rows of young seedlings of tomato, eggplants, and pepper were found to be affected with damping-off, which proved to be caused by *P. debaryanum.*

The beds were dusted with copper carbonate at the rate of 4 grams per square foot as soon as the disease was discovered. A second application of the dust was made a week after the first. Daily observations were made henceforth until the time of removal of the plants to the field. The disease was checked by the treatment.

To check up these results, an experiment was planned in beds 20 ft. × 3 ft. where the soil was a rich loam which had received a light application of cottonseed meal. The soil was infested with cultures of *P. debaryanum* from tobacco. A week later an application of 4 grams of copper carbonate per square foot was well raked into the surface soil. Three beds were employed and each was divided into three subdivisions with partitions which were sunk ten inches below the surface of the soil. The middle portion in the outer beds (1 and 3) and those on the ends of bed No. 2 were left untreated. Eggplant, tomato, and pepper seed was sown in alternate lengthwise rows 4 inches apart in all the beds, except the last section of the third bed, a week after the treatment was made. The order of planting was tomato, eggplant, pepper; and the treated bed sections were planted first and later the checks, so that the rows in one series should correspond with those in the other. There were a total of eleven

rows of tomato and eggplant and 10 rows of pepper in each series; but notes were taken on 10 rows of each. The rows in the check beds were 7 feet long, those in the treated beds, 6.5 feet long; but seedlings were thinned out and only 300 of eggplant and 400 of pepper and tomato were left in each row. Germination was normal in all beds but some of the seedlings in both treated and untreated beds showed symptoms of the disease two weeks after germination. The disease became more serious and a second application was deemed necessary. A week after the first symptoms of the disease appeared, the second application (4 grams per square foot) was made. The disease continued for four days longer when a few seedlings were found to show symptoms. A week later no more seedlings were found dying. However, in the untreated beds the disease increased in severity. Records were taken of the total number of seedlings affected by the disease. Final observations were made when the seedlings were of transplanting age. The results are given in the following table.

TABLE 2

SHOWING RESULTS OF TREATMENT OF SOIL INFESTED WITH *P. debaryanum* WITH TWO FOUR GRAM APPLICATIONS OF CORONA COPPER CARBONATE

Bed Number	Tomato			Pepper			Eggplant		
	Healthy	Diseased	Per cent Healthy	Healthy	Diseased	Per cent Healthy	Healthy	Diseased	Per cent Healthy
1 Treated	1,011	189	84.25	1,107	93	92.25	574	26	95.67
2 Check	75	1,125	6.25	255	945	21.25	136	464	22.67
3 Treated	1,116	84	93.0	714	86	89.25	804	96	89.33
4 Check	93	1,107	7.75	94	706	11.75	114	786	12.67
5 Treated	654	146	81.75	1,155	45	96.25	777	123	86.33
6 Check	86	714	10.75	213	887	17.75	198	702	22.00
7 Treated	602	198	75.25	782	18	97.75	552	48	92.00
8 Check	34	766	4.25	162	638	20.25	154	446	25.67

In order to study the significance of the differences between treated and check beds the results expressed in percentages were paired so that a comparison was established between tomato from the treated beds and tomato from the checks; the same for pepper and for eggplant. Such a study showed that the differences were highly significant, the odds for tomato being well above 4999 : 1, for pepper over 9999 : 1, and for eggplant nearly 9999 : 1, when Student's method was employed. It can be safely concluded from the above data that two applications of 4 grams of copper carbonate applied as described above, will control *Pythium debaryanum* on tomato, pepper and eggplant.

That this treatment was more successful than those on tobacco

may be attributed to the less crowded conditions in the beds with the vegetables. In the latter the sun's rays reach down to the soil at least during the early susceptible stages of the development of the plant and help in drying up the surface layer. This drying up probably also is a factor in disinfestation.

Copper stereate

An experiment was carried out to determine the effect of copper stereate on the control of damping-off. Of fifteen flats (2½ ft. × 3 ft.); five were infested with oatmeal agar mass cultures of P 1, five with P 2, and five with P 3. One flat of each set was left as a check. The remaining four flats of each set were treated one each with 1, 2, 4, and 8 grams of the chemical per square foot, respectively. The seed was sowed a week later. Two weeks after germination damping-off had started in all the flats and therefore a second application was made.

When final notes were taken it was plain that the treatment had been ineffective in controlling *P. Parasitica* (P 1 and P 3) while apparently some control of *P. debaryanum* (P 2) as suggested by healthy seedlings, had been obtained when two applications of 8 grams per square foot had been applied.

A second experiment was conducted in which *P. debaryanum* (P 2) alone served as the damping-off pathogene. Three beds 20 ft. × 3 ft., were employed, of which two were treated with copper stereate at the rate of 8 grams per square foot and the third was left as a check. The seed was sowed a week after the application. Symptoms of the disease were evident ten days after germination, when a second similar application was made. The disease continued unchecked in all the beds and extended to the majority of the seedlings before these had reached transplanting age.

It is concluded that copper stereate is ineffective in the control of the disease.

Uspulun

It was felt that treatment with organic mercury compounds might prove advantageous. Several experiments were made with Uspulun solutions of different concentrations.

In the first experiment three sets of five flats (2½ ft. × 3 ft.) were employed. The soil was infested with P 1, P 2, and P 3 isolates respectively. One flat from each set was selected as a check and the remaining treated each with the following concentrations of Uspulun: 1–300, 1–600, 1–1200 and 1–2400, one week before seed

sowing. The solutions were added in sufficient quantity to soak the soil.

Germination was normal in all flats except those treated with the 1–300 solution in which injury to the seedlings was very evident.

Symptoms of the disease appeared in all flats at an early stage and destruction was complete by the third week in all flats treated with the 1–200 and 1–2400 solutions. A second application was made on the 1–300 and 1–600 flats and this was followed ten days later by a third application. The disease continued unchecked.

The second and third 1–300 applications were injurious to the foliage. The results of this experiment showed that even three applications of a 1–300 Uspulun solution do not control tobacco damping-off.

A second experiment was devised with a 1–400 solution, using different quantities per square foot of surface. Four beds (20 ft. × 3 ft.) previously infested with cultures of the pathogenes, were treated a week before seed sowing and with a 1–400 solution, one each with ¼, ½, and one gallon per square foot, respectively, and the fourth was left as a check.

Germination was perfect in all the beds except that receiving the 1 gallon application, in which very few seedlings developed. The disease appeared in the beds and therefore a second application was made the third week after germination. This did not check the spread of the disease so that at the close of the experiment, three weeks after the second treatment, nearly all the plants had died. (See plate XXXII.)

It may be concluded that a 1–400 Uspulun solution at the rate of 1 gallon per square foot is injurious to seed germination, while at the rate of ¼ or ½ of a gallon it is effective in controlling the disease.

Bayer dust

A preliminary experiment was started with Bayer dust, an organic mercury compound. Four sets of five flats (2½ ft. × 3 ft.) were infested with Pythium and Phytophthora cultures one each with P 1, P 2, P 3, P 10 and P 13. In each set one flat was treated with Bayer dust at the rate of ½ gram, one with 1 gram, another with 2 grams and the fourth with 4 grams per square foot, while the fifth was left as a check. The seed was sown a week after treatment.

Germination was normal in all the flats. The first symptoms of the disease appeared three weeks after germination in all flats. A second application was then made.

The results at the termination of the experiment six weeks after germination, showed that Bayer dust was effective in the control of damping-off even when as many as two applications of four grams per square foot were employed.

The ineffectiveness of this treatment was further shown in an experiment with two four-gram applications of the dust. The beds used were 20 feet × 3 feet and infested with a mixture of oatmeal agar cultures of *P. debaryanum* and *P. Parasitica.* The first application was made a week before sowing the seed and the second was made two weeks after germination. Three beds were treated and one was left as a check. The results showed a complete destruction of the seedlings in the check as well as in the two treated beds. (See plate XXXIII.)

In order to determine if seedlings could be better protected by applications after germination only, the preceding experiment was duplicated using this time the 4 gram treatment only and making two applications, one a week after germination and the other following the first by 10 days.

At the close of the experiment no significant difference between treated and check flats could be found, indicating the non-effectiveness of the Bayer dust.

Copper sulphate

Three beds (20 ft. × 3 ft.) were infested with a mixture of oatmeal agar cultures of P 1, P 2 and P 3 and after a week treated as follows: one with a solution of 2 pounds of copper sulphate in 25 gallons of water, the second with 2.5 pounds in 25 gallons, and the third was left as a check. The third bed was located between the first and second. The rate of application in the two cases was 1 gallon per square foot. Seed was sown a week after application.

Two weeks after germination there was a general occurrence of damping-off in all the beds and the disease continued until only a few scattered plants were left. This indicates the failure of copper sulphate at those concentrations to eradicate the damping-off fungi.

Copper fluosilicate *

Four beds (20ft. × 3 ft.) were prepared as in preceding experiments and infested with a mixture of cultures of *P. debaryanum* and *P. Parasitica.* Three were treated one each with 2, 3 and 4 grams of the dust, respectively, per square foot. The fourth bed was kept as a check. Seed was sown ten days after treatment.

* The copper fluosilicate compound used in this experiment is not manufactured any longer.

A second application of the dust was made three weeks after germination when it was clear that the disease was making headway in all the beds. The dust from the second treatment produced a burning of the foliage of a large number of seedlings but checked the disease in the beds receiving three and four-gram applications. (See plate XXXIV.)

In conclusion, it may be said that copper fluosilicate did not have a deleterious effect on the germinating seedlings. When applied after germination it burned the foliage of the plantlets. Two applications of 2 grams per square foot were not enough to control the disease. Two applications each of 3 and 4 grams of the chemical, one a week before sowing the seed, the other four weeks after seed sowing gave good control.

Acetic acid

Three beds (20 ft. × 3 ft.), where tobacco seedlings had died from damping-off, were treated as follows: No. 1 with one per cent acetic acid, and No. 2 with 1.2 per cent acetic acid, both at the rate of one-half gallon per square foot, while the third bed remained untreated. The concentrations used here are those used by Doran (13) and which he gives as resulting in good control of the damping-off of tobacco.

The seed was sown ten days after treatment.

The observations made after germination showed that the disease appeared twelve days after germination and progressed rapidly until the experiment was terminated when it had spread completely over all the seedlings as in the case of the check bed. The failure of acetic acid to control the disease in Puerto Rico under conditions of high infestation is clear.

Bordeaux mixture

Since Bordeaux mixture had been reported as giving good control in Puerto Rico and Cuba it was deemed desirable to make tests with it under controlled conditions. For this purpose three of the common formulas were used, namely, the 3–3–50, 4–4–50, and 5–5–50. Eight beds (20 ft. × 3 ft.) which were heavily infested with cultures of the pathogenes were treated, two each with each formula and the remaining two were left untreated. The rate of application was one-half gallon per square foot. Seed was sown a week after the treatment.

Germination was excellent in all beds. Two days after germination a few small areas of the disease were observed, and then a

second application at the same rate and formula as the first was made on all the treated beds.

The results show complete damping-off of seedlings in the check beds, about 25 per cent infection in the 3–3–50 beds and about 5 per cent infection in the 4–4–50 and about 5 per cent in the 5–5–50 beds. (See plates XXXV and XXXVI.)

From these results it may be concluded that two applications of 4–4–50 or 5–5–50 Bordeaux mixture, at the rate of ½ gallon per application per square foot, serve as good protectants against damping-off. These applications should be made one before sowing the seed and the other ten to fourteen days after germination.

A further test was made with Bordeaux mixture (4–4–50), on five beds on which the disease had appeared. The diseased areas were treated with 1–30 formaldehyde and then the first application of the bordeaux mixture was made at the rate given in the preceding experiment. The disease continued to spread over the beds so that a second application was made two weeks later.

These beds were exposed to heavy rains so that some of the mixture was undoubtedly washed away.

The results showed that the disease was not completely checked by the two treatments but the treated beds showed a great improvement over the surrounding untreated beds where all the plants were affected.

It may be concluded from these results that applications of Bordeaux mixture (4–4–50) after the disease has made its appearance in a seed-bed do not check completely any further spread though it reduces the amount of disease.

Injury from copper fungicides

In an experiment made in 1927 on beds in the field, where a crop of tobacco seedlings had previously been grown, it was found that injury resulted from an application of cooper carbonate. This is the first case of injury from this compound in any of our experiments. Six beds (120 ft. × 4 ft.) had been treated with the dust at the rate of four grams per square foot, one week before seed sowing. The beds were well watered during all the time the experiment lasted. It should be added that the neighboring beds, making a total of about one acre, were treated with 1–50 formaldehyde at the usual rate.

The injury in the copper carbonate-treated beds was manifested in much delayed germination and the few seedlings that developed

were stunted and yellow. No injury resulted from the formaldehyde treatment under the same conditions.

The injury of the compound was not due to a too heavy application since it was the same as employed in all previous experiments with the same dust, in which no injury ever resulted. It can not be attributed to dryness since the beds were liberally watered twice a day.

An experiment was planned in 1928 in order to find out whether such deleterious action could be prevented. The conditions of the preceding experiment were duplicated as nearly as possible. A seedbed, on the same kind of soil (a heavy loam), which was growing a crop of tobacco seedlings was selected. It was assumed that the injury occurred either because too much copper became soluble or because some compound or substance was formed on these soils which was injurious to germination. The beds were cleaned in December, the soil reworked, and an application of a 6–7–8 fertilizer (nitrogen in the form of sulphate of ammonia and cottonseed meal) applied.

Besides copper carbonate, Bordeaux mixture was also used in the experiment. As checks to the copper disinfestants, acetic acid and formaldehyde were employed, the latter because no injury had followed this treatment on the same soil where copper carbonate had been injurious and the former because of the change in soil reaction which would be expected with its use. Charcoal was used as an absorbent.

The beds were 40 ft × 4 ft. and they were protected from insolation by a cheese-cloth shade. There was very little rain during the time the experiment was in progress so that it may be said that all the beds alike received a moderate amount of water. Watering was done once a day, in the morning. Soil samples for the determination of active acidity were taken a week before the application of the fungicides and again at the time injury first appeared in some of the beds.

The charcoal used in the experiment was very finely ground; it was applied at the rate of 1 ounce per square foot of surface on January 8. The disinfestants were applied January 11 at the following rates per square foot; copper carbonate, four grams; Bordeaux mixture (4–4–50), ½ gallon; acetic acid (1.2 per cent), ½ gallon; and formaldehyde (1–50), ½ gallon. Both the charcoal and the copper carbonate were incorporated by raking them well into the loose surface soil. The liquid solutions were applied with sprinkling cans.

The seed was sown in all beds January 24, 1929. The arrangement of beds, treatments, and results appear on Table 3.

TABLE 3

SHOWING EFFECTS OF CHARCOAL ON COPPER CARBONATE AND BORDEAUX MIXTURE - TREATED BEDS; ALSO EFFECT OF ACETIC ACID AND FORMALDEHYDE; SOIL OF OLD BEDS

Beds	Treatment	Results
1	Check	Normal development
2, 3	Charcoal alone	Normal development, slightly lower than check
4, 5, 6	Copper carbonate plus charcoal	Yellowing of seedlings; about 25 per cent of plants
7, 8, 9	Copper carbonate alone	Yellowing of seedlings; about 25 per cent of plants
10, 11	Checks	Normal development
12, 13, 14	Bordeaux (4–4–50) plus charcoal	Slight yellowing on about 5 per cent of plants
15, 16, 17	Bordeaux (4–4–50) alone	Slight yellowing on about 5 per cent of plants
18, 19	Checks	Normal development
20, 21	Acetic acid (1.2%) plus charcoal	Normal development; like checks
22, 23	Acetic acid (1.2%) alone	Normal development; growth more rapid than in check
24	Check	Normal development
25, 26	Formaldehyde plus charcoal	Normal development
27, 28	Formaldehyde alone	Normal development; more rapid than in checks; similar to beds treated with acetic acid
29, 30	Charcoal alone	Normal development; slightly slower than checks
31	Check	Normal development.

It is plain that charcoal did not prevent the injurious action of copper carbonate and Bordeaux mixture. The fact that Bordeaux mixture was also injurious to seedlings grown under the same conditions as those of beds treated with copper carbonate or with no treatment at all, seems to indicate that copper is the toxic agent. Beds treated with formaldehyde and those with acetic acid were not only normal but the stand of seedlings and rapidity of growth surpassed that of the non-treated beds. Development in the beds treated with charcoal alone was slightly slower than that of the non-treated beds. This action of the charcoal was evident when beds treated with acetic acid or formaldehyde, with and without charcoal were compared. Growth was much more rapid where charcoal was not used.

It seems likely that the cause of the injury by the copper compounds can be attributed to some chemical reaction with the soil. The object of this experiment has not been to determine, through the proper chemical procedure, the real nature of the toxicity in question. Therefore, we shall point out only the probable cause or causes which the results in table 3 suggest. The favorable effect of formaldehyde and acetic acid as contrasted with the rather injurious tendency of copper carbonate and Bordeaux mixture, suggests that the latter treatments have alike induced a certain reaction to which may be attributed the injury produced on seedlings. The pH reaction of the soil may directly have little to do with these results. On discussing the general effect of the various treatments we can

draw the following considerations. The disinfesting action of formaldehyde and acetic acid is only temporary. Therefore, as they do not prevent secondary infestations their use is undesirable. It is obvious that the beneficial soil flora will regain its predominance without much difficulty. On the other hand, the disinfesting properties of copper carbonate and Bordeaux mixture are more lasting and it may be possible that the nitrifying organisms in the soil may cease to increase in numbers and their action be reduced. Were this to occur the whole question might be referred to nitrification. The condition of the seedlings would suggest starvation of some kind. Now, according to this assumption, the injury in the beds treated with the copper compounds occurs because nitrification is prevented. In those not so treated or where either acetic acid or formaldehyde were applied, nitrification proceeded after the treatment. That this injury does not occur in new land or in soils not previously growing a tobacco seed-bed, may be given further in support of this assumption. A soil in which a tobacco seed-bed has been growing is almost depleted of its nitrates as evidenced from the failure of a second crop immediately following the first. The success of the second seed-bed, then, depends entirely on the nutrients rendered available during the time it is in progress. If any difficulty arises whereby these nutrients are prevented from being incorporated in an available form, the failure of the crop may be expected.

In an experiment conducted in flats in the greenhouse in the fall of 1931 injury from Bordeaux mixture was again observed. Two flats were sprayed weekly for three weeks with a sodium nitrate solution (1 ounce to 1 gallon). The majority of the seedlings recovered and made normal growth. No further experiments were carried out and these results must, therefore, stand only as a suggestion for more controlled experiments.

In connection with injury resulting from copper it may be added here that Mme. Vladimirskaya (30) in studies on the action of various methods of soil disinfestation upon the physical and chemical constitution of the soil, found that among other substances copper carbonate had a deleterious effect on nitrification. She further found that formaldehyde at first suppressed but later stimulated the development of the nitrifying flora of the soil. Our results and observations appear to be in line with her results.

Summing up, in our opinion it may happen that the fertilizer added to a soil which is later treated with a disinfestant of lasting effect, is not rendered available and therefore the seedlings can make no use of it.

Effect of cultivation

It was thought that constant cultivation for a period of five or six months might reduce the amount of infestation in a soil. If possible this would be a practical means of eradication which farmers in Puerto Rico would not hesitate in putting into effect.

An experiment was started in 1928 with a view of ascertaining the effect of cultivation. Two beds, where some chemical treatments had been unsuccessful, were chosen because these were known to be heavily infested. In one of the beds subdivided into three sections, copper sulfate and Uspulun solutions had failed to control the disease and in the second bed Bayer dust and mercuric chloride had been unsuccessful in experiments which lasted until March 26. A third bed which had had damping-off all the time was selected as a check. The size of the beds was 20 ft. × 3 ft.

This experiment was begun on April 15. The three beds were sown on that date, because we wanted to know the degree of infestation of the beds at the time the experiment was commenced. The germination in the beds was excellent but damping-off began to appear on the young seedlings soon after they emerged. All the seedlings had been killed by May 10, when two of the beds were weeded and the soil loosened up well with a spade and rake. The third bed was left unweeded throughout the time the experiment lasted. The two treated beds were weeded twice a month when the soil was again loosened up. This continued until Nov. 15, when the check bed was also weeded and the soil put in shape for the sowing of the seed. There was good germination in all the beds. Damping-off appeared in the three beds simultaneously on Nov. 26, and spread very rapidly. On Dec. 6 more than 76 per cent of seedlings had been killed in each of the three beds and destruction was complete by Dec. 10.

The results show that about six months of constant cultivation of an infested soil does not reduce the amount of infestation of the damping-off pathogenes.

To test whether the treatment would be efficacious over a period longer than six months, the same beds were similarly treated from Dec. 10, 1928 to Dec. 10, 1929. The seed was again sown. The disease appeared again. When the experiment was closed, i. e., when plants were of transplanting size, not less than 60–70 per cent of the stand had been destroyed by the disease, which shows that even a year of continuous cultivation fails to eradicate the disease.

This experiment was not conducted over a longer period because

it was realized that even one year of such a treatment would be highly impractical in Puerto Rico.

PROTECTION

The experimental part on protection has been given with that on eradication. In tobacco damping-off, it appears that a definite, clearly cut line cannot be drawn between protection and eradication. They are, in our minds so linked to each other that a discussion of the data separately would detract very much from the value and meaning of the same.

With the eradication of the damping-off pathogenes naturally goes the destruction of the majority of the organisms constituting the soil flora, thus breaking the equilibrium which must normally exist among these soil inhabitants. A reinfestation by the pathogenes causing damping-off may rapidly gain a foothold in the new habitat, the fungi spreading with much rapidity. Tobacco seedlings, under the conditions of moisture and temperature characteristic of those overcrowded plant populations, and with their high susceptibility offer these pathogenes the most favorable abode. It is clear that some means of insuring the best development of the seedlings must be provided. A barrier, therefore, must be erected between the susceptible plant parts (leaf, stem, and roots of seedlings) and the parasite. This may consist of some substance which when applied to the parts susceptible of penetration will kill the inoculum. Such a substance may also be applied to the environment where the pathogene is harbored or where it may extend into, checking its progress there. This aim might also be attained by modifying other external conditions or factors which influence the development, spread and severity of the disease.

Methods of protection to follow those of eradication have already been discussed in relation to the disinfestation experiments. It was found that copper dusts or compounds when applied to the soil and plants will protect the latter considerably from new attacks. The best methods of control are those which combine the means whereby the amount of inoculum in the soil is reduced to the minimum and which insures a relative amount of protection thereafter.

A discussion of other protective measures for seed-beds against damping-off may be found in the literature. The application of a layer of dry or hot sand to the surface of the beds after the seed is sown will give good results in connection with the damping-off of other seedlings like citrus and vegetable garden crops; but with to-

bacco, the seed of which is very small, it will probably be inapplicable, aside from being too costly on a large scale. It seems that the avoidance of organic matter, especially fresh barnyard manure, will do much good in preventing the disease. Yet a clean bed when enriched by the addition of uninfested manure will be as safe as any other soil, and, besides, will give strong, rapidly developing plants. Any uninfested seed-bed may be protected from infestation by digging a wide trench or ditch on all sides and especially on that side from which the drainage water is expected to run. This ditch must be wide and deep enough to take care of all the superfluous surface water during heavy rains. It should drain away from the seed-bed, and under no circumstances should lateral ditches be allowed to drain through the beds.

Great damages will be prevented sometimes by clean and careful culture. Weeding should be done as carefully as possible. There is often more harm resulting from careless weeding than the good which should follow the removal of the weeds. Before proceeding to weed a field a careful inspection of the bed should be made and wherever symptoms of the disease are detected the diseased areas should be drenched with a 1–30 formaldehyde solution. This done, the beds may be weeded without danger, unless the pathogenes have already spread too far away from the treated areas at the time these were detected. It is always a good practice to apply the disinfestant even beyond the zones of infection. Persons handling diseased plants should wash their hands in a disinfecting solution, then in water before going into healthy beds. Any tools used in such beds should be sterilized in a formaldehyde solution before using them in other beds. Wet, low places in seed-beds are usually a source of inocula. The disease will first appear here and the inoculum is then transported by the many agents into other beds. All such wet spots should be avoided when looking for a suitable site for the beds. When they cannot be avoided they should be drained well and the drainage ditch should empty into the outside. Pools of water should not be allowed to form in the ditches because they are often the sources of inocula.

Summary

1. Damping-off of tobacco is a very severe disease in Puerto Rico.

2. The disease is caused by *Pythium debaryanum* and *Phytophthora Parasitica* var. *nicotianae*.

3. The agents of transportation of the fungus are water currents, laborers, animals, burrowing insects, etc.

4. Leaves are infected by zoospores of *Phy. Parasitica* var. *nicotianae* but apparently not by those of *P. debaryanum.*

5. Environmental conditions are important factors influencing the spread and severity of the disease. The disease seems to be equally severe during all seasons provided the proper moisture relations are maintained. Organic manures seem to influence favorably the incidence of the disease. The disease is severe on thickly-sowed beds.

6. Control of damping-off of tobacco is today one of the most serious problems with Puerto Rico tobacco growers.

7. When the disease appears in small areas only, it may be checked by drenching these with a 1–30 formaldehyde solution.

8. Soil disinfestation by means of steam or with formaldehyde does not seem to be practicable under Puerto Rican conditions.

9. *Phy. Parasitica* var. *nicotianae* is probably slightly less susceptible to the sterilizing action of formaldehyde than *P. debaryanum.*

10. Mercury compounds have been found injurious to tobacco seedlings, and ineffective against the damping-off pathogenes.

11. In preliminary trials two applications of Corona Copper carbonate of four grams per square foot, before seed sowing, and at the same rate a week after germination, were fairly effective.

Two applications of copper carbonate in the field did not give effective control probably due to the heavy rains and to overcrowding of the seedlings. Two late applications of copper carbonate on heavily infected beds were unsuccessful. Two 4-gram applications of copper carbonate resulted in good control of the damping-off of tomato, pepper, and eggplant.

12. Copper stereate, in two applications of 4 grams each, seemed to control *P. debaryanum* but did not have any effect on *Phy. Parasitica* var. *nicotianae.*

13. Bayer dust and Uspulun were injurious when applied to the foliage and proved to be ineffective in the control of the disease.

14. Copper sulfate solutions (4 and 5 pounds to 50 gallons), applied at the rate of ½ gallon per square foot before sowing the seed were ineffective.

15. Effectiveness of copper fluosilicate is doubtful.

16. Acetic acid does not control the disease under conditions of high infection.

17. Two applications of 4–4–50 and 5–5–50 Bordeaux mixture at the rate of ½ gallon per square foot, one before sowing the seed and the other a week after germination, were effective in controlling

damping-off. The treatment was not very successful when applied to beds in the field in which the disease had made its appearance.

18. Injury to seedlings resulted when copper carbonate was applied to a tobacco seed-bed on the site of an old bed. It was proved by experiment that the injury was not due to dryness. Soil reaction appears to have little to do as a direct cause of the injurious action. No injurious action of formaldehyde or acetic acid was found under similar conditions. Charcoal was not effective in preventing injury from the copper compounds. Recovery from injury resulted in one case when a sodium nitrate solution was applied. It is suggested that the injurious action is connected with nitrification which would be hindered by the lasting effect of the copper treatments.

19. Continuous cultivation of infested soils for periods of six to twelve months does not eradicate the disease.

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Explanation of Plates

Plate XXVII. Healthy tobaco seedlings.

Plate XXVIII. Tobacco seedlings showing lesions produced by infection with *Phythium debaryanum.* Note that they are confined to the region of the stem at the soil surface.

Plate XXIX. Tobacco seedlings infected with *Phytophthora Parasitica.* Note that the lesions may occur higher up the stem than the surface of the soil.

Plate XXX. Bed treated with four grams of copper carbonate (Corona) at the time of seeding and two weeks after germination.

Pate XXXI. Another bed treated with four grams of Corona copper arbonate a week before seeding and two weeks after germination.

Plate XXXII. Bed treated a week before sowing seed with a 1–400 Uspulun solution at the rate of one-half gallon per square foot of surface.

Plate XXXIII. Bed treated a week before sowing the seed, with Bayer dust at the rate of 2 ounces per square yard. Note the complete destruction of seedlings with only a few at the lower corners surviving.

Plate XXXIV. An application of four grams of copper flousilicate made after the appearance of the disease resulted in good control though in injury to many seedlings.

Plate XXXV. Two applications of Bordeaux mixture (4–4–50) at the rate of one-half gallon per square foot, one at the time of seeding, the other two weeks after germination. Note the stand and vigor of the seedlings.

Plate XXXVI. Two applications of 5–5–50 Bordeaux mixture, one at the time of seeding, the other two weeks after germination. Rate of application, one-half gallon per square foot of surface.

Plate XXXVII. An infested bed from the checks. Note the almost complete destruction of seedlings. The majority of the seedlings showed infection at the time the photograph was taken.

PLATE XXVII

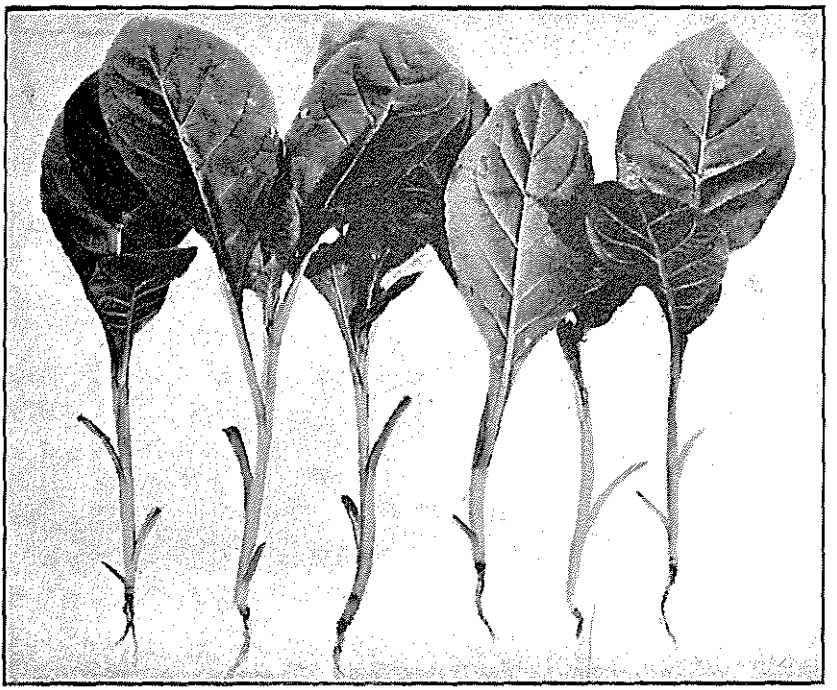

PLATE XXVIII

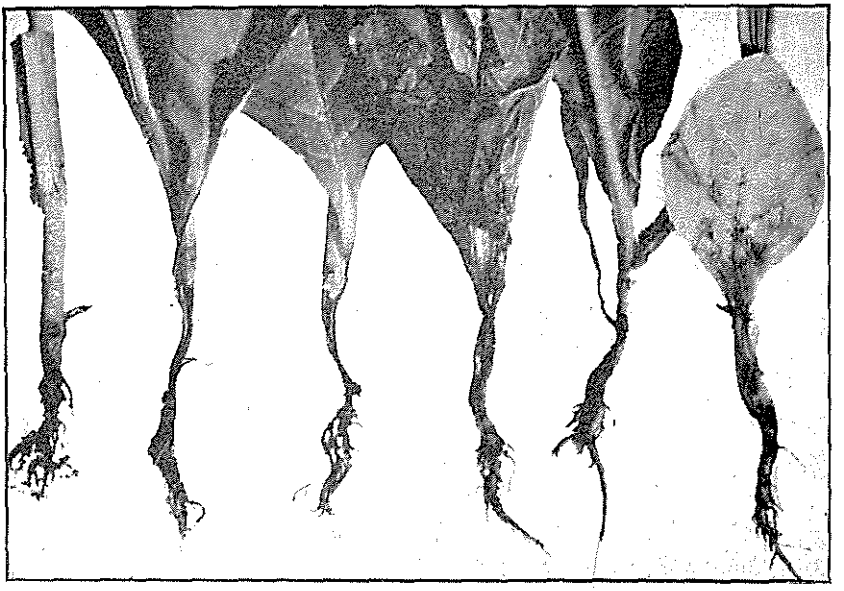

PLATE XXIX

PLATE XXX

PLATE XXXI

PLATE XXXII

PLATE XXXIII

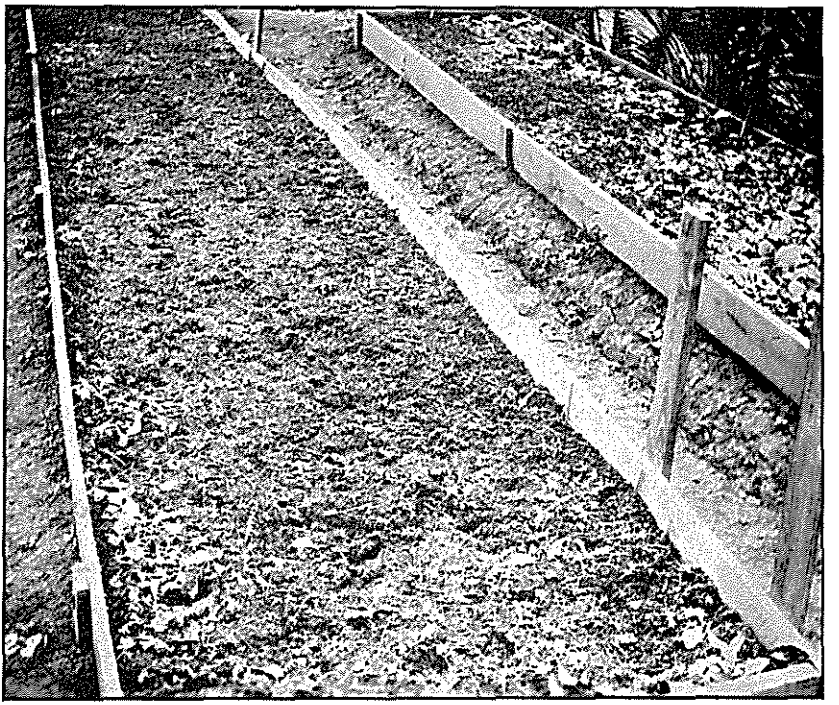

PLATE XXXIV

PLATE XXXV

PLATE XXXVI

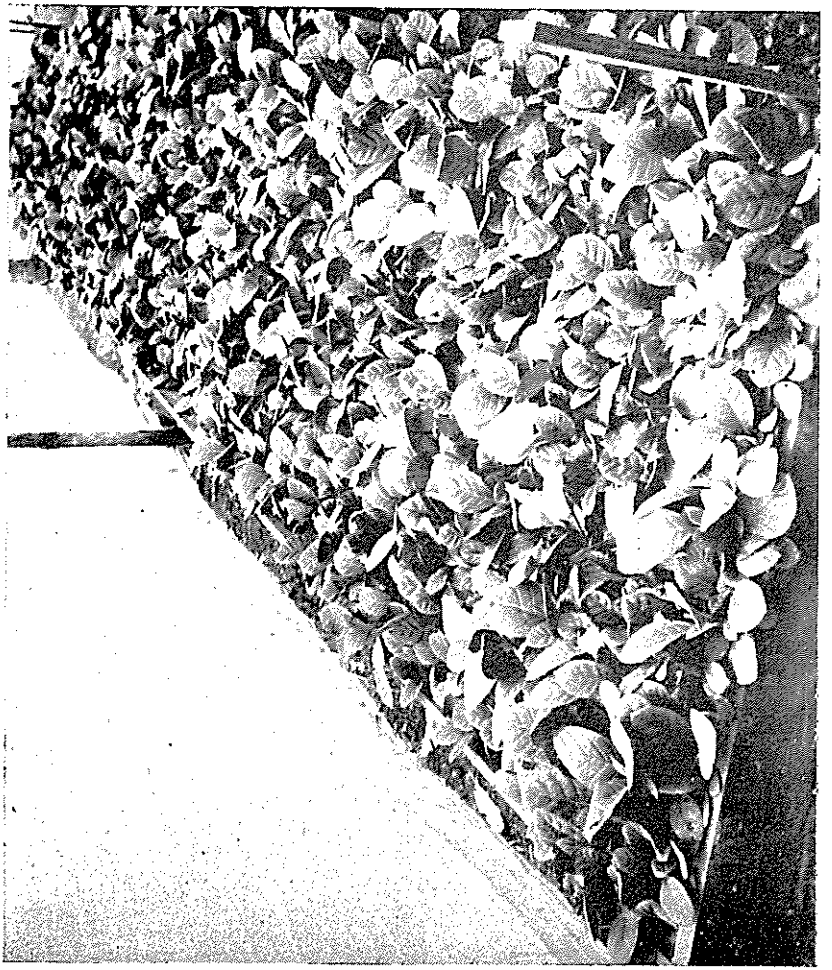

PLATE XXXVII