EXPERIMENTS ON THE CONTROL OF SEED-BORNE DISEASES BY X RAYS

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INTRODUCTION

Seed-borne diseases are those whose causal organisms are carried either within, or upon the surface of, the dormant seeds. The plants from infected seeds may develop serious seedling infections or, in case the disease is systemic, disorders in the mature plants. There are a number of crop seeds which carry the causal organisms of economically important diseases.

The losses resulting from seed-borne diseases vary, depending on the areas under consideration and the care used in seed selection. In some cases they have been estimated at as much as 10 per cent of the crop. Among the organisms that infect corn, four were selected for the purpose of this study: Diplodia zeae (Schw.) Lév., Gibberella saubinetii (Mont.) sacc., Fusarium moniliforme Sheld., and Cephalosporium acremonium Cda. Of these D. zeae and G. saubinetii are probably the most serious, especially from the standpoint of corn-seedling injury. Other organisms studied were loose smut, Ustilago tritici (Pers.) Rostr., and bunt, Tilletia levis Kühn, of wheat; loose smut, U. nuda (Jens.) Kell. and Sw., of barley; and loose smut U. avenae (Pers.) Jens., of oats.

The recommended control of these organisms includes the application of germicides to the seed surface, and temperature treatments. Carefully conducted tests have indicated that organic mercury compounds are valuable in the control of certain organisms attacking corn. Copper carbonate and formaldehyde are widely used in the control of certain cereal diseases, and hot-water treatments have been used successfully in controlling loose smut of wheat.

The problem of controlling seed-borne organisms with X rays involves a study of the host reaction as well as that of the parasite. The X-ray treatment should not injure the host. Additional practical considerations are the efficiency of this treatment as compared with others and the cost. The use of X rays would permit treatment at any time before planting and would not unfit the seed for food purposes as do many chemicals.

REVIEW OF LITERATURE

The results obtained by using X rays as the sterilizing agent have varied. Much work has been done on the treatment of bacteria, especially those parasitic on man. Only those references dealing with the irradiation of plants will be cited.


(909)
Trumbull and Hotson (12) tried X rays as a means of controlling *Fomes pinicola* (Fr.) Cke. on logs of fir and western hemlock, but without success. Levin and Levine (5) inoculated a series of castor beans, *Ricinus communis*, with *Bacterium tumefaciens* EFS. and Town. and immediately afterwards subjected the inoculation site to X rays. Six treatments given at intervals of two days prevented the development of tumors, apparently with no injury to the host plants. Rivera (9) described the successful control of *Bact. tumefaciens* tumors on castor-beans and geranium plants. In a later paper (11) he concluded that the preventive action was a function of the effect of radiation on the host tissue rather than on the bacterial cells. Nadson and Philippov (7) found the vegetative tissue of Mucor more resistant to X rays than were the sporangia. Small doses appeared to stimulate the development of *M. genevensis*.

Rivera (10) irradiated cereal seeds heavily infected by smut fungi. The treated cereals produced 36.1 per cent infected spikelets, and the untreated produced 36 per cent. Pichler and Wober (8) obtained favorable control results with spores of *Tilletia tritici* (Bjerk.) Wint. The killing action was augmented by increasing the acidity of the medium in which the spores were irradiated, particularly in the presence of oxygen or oxygen-yielding substances. Irradiated seed appeared to be injured slightly by the treatments, but potato tubers treated for the control of *Chrysophlyctis endobiotica* Schilb. were not injured. Preliminary observations by Mulvania (6) showed that X-ray treatments on the virus of tobacco mosaic had no apparent effect. Johnson (4) found that *Sclerotium bataticola* Taub., *Collybia dryophila* Fr., and *Fusarium batatatis* Wr. were unaffected by X-ray treatments.

**EXPERIMENTAL PROCEDURE**

In the study reported in this paper two phases of the problem of controlling seed-borne diseases by X rays were considered—the reaction of pure cultures of the fungi, and the reaction of the host and associated parasite to X rays.

The pure cultures were obtained from the botany department of the University of Missouri, with the exception of *Diplodia zeae* which was isolated from a corn seed.

Streak transfers were made to thin layers of potato-dextrose agar in Petri dishes. The cultures were incubated from three to five days before irradiation. Immediately before irradiation the colonies in each Petri dish were cut into small squares (3 to 4 mm). During irradiation the Petri dish covers were removed and replaced by a washed photographic film. The four fungi, each in a separate Petri dish, were irradiated simultaneously. After treatment the squares were removed from the culture and transferred to a Petri dish containing potato-dextrose agar. The effects of irradiation were noted after incubating the squares eight days at 28° C.

An old model Kelly-Koett X-ray machine was used. Intensity measurements were made with a Victoreen iontoquantimeter. A description of the dose given in each treatment includes the peak voltage, milliamperage, distance of the target from the material being X rayed, the length of exposure, and the dose in International r units per minute.

Lots of corn seed infected with *Diplodia zeae* and *Gibberella saubinettii* and nearly disease-free seed were obtained from Dr. J. R.
Holbert, Bloomington, III. The corn seeds for the isolation experiments were treated, germ side up, in a layer one seed deep. Those for the field experiments were exposed at random in a layer approximately three seeds deep.

The presence of internal parasites in the corn seeds was investigated by germinating the seeds on standard potato-dextrose agar at 28° C. The method used was similar to that of Chen (1). The field plantings were in a fertile silt loam. The rows were 30 inches apart and the seeds 6 inches apart in the row.

The infected wheat, barley, and oats were secured from the Missouri experiment station.

**TREATMENT OF PURE CULTURES OF FUNGI**

Preliminary experiments with pure cultures of *Penicillium* sp., *Aspergillus* sp. and *Diplodia zeae* showed that exposure to X rays decreased the rate of growth of the fungi and that large doses caused death or inactivation. However, the doses required to kill the fungi in pure cultures seriously injured dormant corn seeds.

Cultures of *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium acremonium* were X rayed at different temperatures in order to establish their lethal doses and to determine the effect of temperature on the lethal doses. The cultures were exposed to the respective temperatures for 10 minutes preceding the treatment and during the period of treatment. Table 1 summarizes the effects of X rays, on the mycelium and spores of fungi at various temperatures.

**Table 1.** Effect of X rays on mycelium and spores of fungi in culture at various temperatures as determined by the development of X-rayed transfers

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Fungus</th>
<th>Development after being X rayed for indicated period in minutes *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  30  40  50  60  70  90  100  110  130</td>
</tr>
<tr>
<td>10</td>
<td><em>Diplodia zeae</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<tr>
<td></td>
<td><em>Gibberella saubinetii</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<tr>
<td></td>
<td><em>Fusarium moniliforme</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<tr>
<td></td>
<td><em>Cephalosporium acremonium</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
</tr>
<tr>
<td>20</td>
<td><em>Diplodia zeae</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
</tr>
<tr>
<td></td>
<td><em>Gibberella saubinetii</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<td></td>
<td><em>Fusarium moniliforme</em></td>
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<td></td>
<td><em>Cephalosporium acremonium</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<tr>
<td>30</td>
<td><em>Diplodia zeae</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<td></td>
<td><em>Gibberella saubinetii</em></td>
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<tr>
<td>40</td>
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<td></td>
<td><em>Gibberella saubinetii</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<td></td>
<td><em>Fusarium moniliforme</em></td>
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<td></td>
<td><em>Cephalosporium acremonium</em></td>
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</tr>
<tr>
<td>50</td>
<td><em>Diplodia zeae</em></td>
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</tr>
<tr>
<td></td>
<td><em>Gibberella saubinetii</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<td></td>
<td><em>Fusarium moniliforme</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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<td></td>
<td><em>Cephalosporium acremonium</em></td>
<td>-    -    -    -    -    -    +    +    +    +</td>
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</table>

* Plus sign indicates growth; minus sign no growth.

The resistance of *Fusarium moniliforme* and the susceptibility of *Diplodia zeae* indicate a wide range in the tolerance of different fungi. The order of susceptibility to X rays was *D. zeae*, *Gibberella saubinetii*, *Cephalosporium acremonium*, and *F. moniliforme*. 
The susceptibility of the fungi to X rays was increased by exposure to high temperatures. Cultures of the fungi used showed no pronounced effects of exposure to the temperatures alone. It is probable that the cultures X rayed at the extreme temperatures were not at the designated temperature throughout the X ray period because of the short pretreatment exposure of 10 minutes.

_Diplodia zeae_, when X rayed at 10° C. resumed growth after several days. After three days' incubation only a trace of mycelial development was noted in each of the cultures. Two days later large colonies had developed in those receiving 70 and 90 minute treatments, but there was little or no further development in the remaining cultures. Five days later the latter showed definite growth.

Of the cultures X rayed at 20° C. only the culture receiving the 70-minute treatment resumed growth at the end of the 10-day observational period.

The fungi used grew as well on X-rayed agar as on the unirradiated in these tests. However, it is possible that the X-ray treatment affected the medium on which the cultures had been growing, thus accounting wholly or in part for growth differences.

_Diplodia zeae_ did not develop spores after a 4-day incubation at 28° C., while the remaining three fungi produced fruiting bodies to some extent. Chlamydospores were produced by _Gibberella saubinetii_. The presence of occasional resistant spores in these cultures may have accounted for the growth in the culture receiving the 70-minute treatment at 40°.

**TREATMENT OF INFECTED CORN SEEDS**

Corn seeds infected by _Diplodia zeae_ and _Gibberella saubinetii_ were used, since these fungi are most susceptible to X rays in pure cultures and are of more economic importance.

X-ray treatments (120 k. v. (peak), 4 ma., 16 cm target distance, 80 minutes, 375 r units per minute, 50° C. temperature) in the dormant stage of infected seeds did not kill the seed-borne fungi as shown by the vegetative growth made on potato-dextrose agar. This dose injured the corn seeds severely. The fungi in pure cultures are more susceptible than those in the dormant host. This is probably on account of the inactive and resistant form of the parasite and the partial absorption of the X rays by the seeds.

Treatments designed to increase the susceptibility of the parasite without increasing that of the host were attempted. Dormant corn seeds were exposed three days to air saturated with water vapor at 30° C. The control of _Diplodia zeae_ was not affected by an X-ray dose distinctly injurious to the host. Corn seeds soaked for periods of 15, 30, 60, 120, and 240 minutes were not freed of _D. zeae_ by an X-ray treatment sufficient to cause injury to the seed. Increasing the moisture content of the corn seeds to 30 per cent did not markedly increase the susceptibility of the parasite. The degree of infection resulting from _D. zeae_ appears to decrease to some extent in X-rayed seed corn, with storage.

**FIELD TESTS**

In order to test further the effects of X rays on seed-borne organisms dormant corn seeds infected with _Diplodia zeae_ and _Gibberella saubinetii_ were X-rayed and planted immediately in the field. Seeds that had received an organic mercury treatment, as recommended
by Holbert, Reddy, and Koehler (3), were also planted in order to compare the effects of the two methods of treatment.

Forty kernels were used in each experiment and were planted 1 foot apart in the rows. Four plantings were made at weekly intervals. Infected seedlings were identified by examination of the roots and mesocotyls.

Control of the seed-borne diseases was not effected by the range of treatments used. Total doses of from 375 to 12,000 r units showed no effect except a definite decrease in plant growth when the larger doses were used. X rays having a range of wave lengths obtained by voltages of 56, 78, 120, and 120 k. v. filtered through 1 mm of copper had no effect on the percentage of infection. Intermittent treatments given at weekly intervals did not add to the preventive action. The organic mercury treatment decreased Diplodia zeae infection approximately 50 per cent but had no effect on Gibberella sabbinetii. There was no evidence of stimulation in any of the treatments. The diseased plants were distinctly inferior to those produced by the nearly disease-free corn.

TREATMENT OF INFECTED CEREAL SEEDS

Wheat seeds infected with Ustilago tritici were X rayed (107 k. v. (peak), 4 ma., 16 cm target distance, 370 r units each minute, seeds 1 cm deep) in the dormant and germinating stages. A treatment of 30 minutes in the dormant stage reduced the percentage of infection to approximately one-third that of the untreated seeds and noticeably injured plant growth.

Germinating wheat seeds (soaked 12 hours, aerated 12 hours) were X rayed 5 minutes (375 r units each minute). Complete control of the fungus resulted, but the plants received serious injury. A 2½-minute treatment decreased infection approximately 50 per cent and also injured the plants. An equivalent treatment, a ¼-mm copper filter being used, reduced infection to approximately one-eighth that of the check without serious injury to the plants.

Dormant wheat seeds infected with Tilletia levis were treated similarly. Infected heads were observed in the plants that had received the 40-minute treatment. The percentage of infection of the untreated lot was too small to determine the extent of control.

An X-ray treatment (140 k. v. (peak), 4 ma, 24 cm target distance, 240 r units each minute, seeds 1 cm deep) of dormant barley seeds for 68 minutes slightly reduced the infection of Ustilago nuda and noticeably reduced the stand and vigor of the plants. A 16-minute treatment of germinating barley seeds (soaked 8 hours, aerated 12 hours) effected complete control but reduced the stand to less than 5 per cent that of the untreated seeds.

X-ray treatments applied to oats in the germinating stage were more effective in the control of Ustilago avenae than were those applied to the dormant oat seeds. Doses (140 k.v. (peak), 4 ma., 24 cm target distance, 240 r units each minute, seeds 1 cm deep) applied for 17 minutes to dormant seeds reduced infection approximately 50 per cent. An X-ray treatment of 68 minutes did not reduce infection further. Infection was reduced approximately 25 per cent in the oats treated in the germinating stage (soaked 4 hours, aerated 4 hours) for 33 minutes, but the mature oat plants were slightly injured. This suggests that some of the organisms are more susceptible to X rays.
These treatments indicate that the differentials used in the killing points of the hosts and parasites are not large enough to allow control without serious injury to the hosts. Germinating the seeds affected the differential favorably. The complete control effected in the case of loose smut of barley suggests the use of X rays in investigations where chemical residues are not desired, and where a fraction of the original host population is sufficient.

Negative results secured by other workers, where comparisons could be made, may be ascribed to too short a period of treatment with X rays.

SUMMARY

The control of seed-borne diseases by X rays depends on the differential in the killing points of the hosts and their parasites. This differential was found not to be sufficient to permit the complete control of the organisms studied.

While Diplodia zeae was quite susceptible to X rays in the actively growing cultures, it was extremely resistant in the dormant corn seed. This probably is due to the inactive condition of the organism, the presence of resistant spores, and the absorption of radiant energy by the seeds. An X-ray dose several times the lethal dose for corn was required to affect the organism.

The decreasing order of tolerance of the fungi used in culture was: Fusarium moniliforme, Cephalosporium acremonium, Gibberella saubinetti, and Diplodia zeae. The possible presence of spores in all but D. zeae at the time the fungi were X rayed may account for their greater resistance.

Supplementary treatments used in an attempt to modify the fungus or host in order to obtain a more favorable difference in killing points were not successful. The treatments affected the host and parasite similarly.

The organic mercury treatment was effective in partially controlling Diplodia zeae but appeared to be ineffective with Gibberella saubinetti. X-ray treatments of dormant wheat, barley, and oat seeds infected with Ustilago tritici, Tilletia levis, Ustilago nuda, and U. avenae, respectively, did not effect control.

X-ray treatments of germinating seeds significantly decreased the percentage of loose smut of oats and completely controlled loose smut of barley, although germination was decreased to as low as 5 per cent in the barley.

Seeds as well as fungi vary widely in their lethal X-ray doses. It is possible that seeds requiring large lethal doses may be infected by fungi requiring small lethal doses, in which cases control by X rays may be effective.

LITERATURE CITED


