STERILIZATION OF CORN GRAINS WITH SODIUM HYPOCHLORITE

RAYMOND E. GIRTON

(WITH FOUR FIGURES)

Introduction

Before carrying out a respiration study on detached roots, it was necessary to work out a method for obtaining root tissues free from microorganisms. This was essential because fungi give off considerable carbon dioxide in respiration; and the production of respiratory carbon dioxide by aseptic roots may be entirely masked by the presence of these microorganisms.

Following the method outlined by Wilson (4), an aqueous extract was prepared from bleaching powder. Corn grains were treated with this solution, and allowed to germinate on sterile agar, as employed in the method of Robbins (2). Other grains were treated with a 1 per cent organic mercury preparation, and germinated on sterile agar. Neither of these methods proved to be satisfactory. Since roots which grow from seed sterilized with the organic mercury solution frequently become infected after being detached from the seed, it was assumed that some of the microorganisms were not killed, but were merely inhibited by the disinfectant. A volatile disinfectant, sodium hypochlorite, was therefore chosen because it would either kill the fungi and bacteria on the corn grains during the period of treatment, or allow the subsequent growth and detection of the microorganisms before the roots were detached. Duggar and Davis (1) included sodium hypochlorite in their list of substances tested as seed disinfectants. They used solutions made up from solid (?) sodium hypochlorite, and obtained favorable results which, however, were not altogether uniform.

Preliminary procedure

STANDARDIZATION OF DISINFECTING SOLUTION

A commercial preparation of sodium hypochlorite in solution was obtained. The effective chlorine strength of this solution was determined by a method outlined by Treadwell and Hall (3). This method is based upon the assumption that the oxidation of combined iodine to free iodine is brought about by the chlorine in the disinfecting solution. The iodine thus released is titrated with sodium thiosulphate. Starch is used as the indicator.

The free chlorine of the commercial hypochlorite solution was estimated as 3 per cent. An appropriate dilution of this solution reduced the chlorine concentration to 1 per cent., which was an effective strength for the sterilization of corn grains.
Sterilization of grains

The sterilization procedure consisted in treating about 100 grains of corn in a stoppered flask with 50 cc. of the disinfecting solution. The length of treatment was from 0.5 to 5 hours. During this period, the flask was continuously shaken to promote thorough mixing of the solution and effective sterilization.

At the end of the treatment, the stopper was partially removed and the hypochlorite solution drained off. The grains were then transferred, by means of a long handled metal spoon, to Petri dishes which contained sterile agar. The spoon was frequently dipped into alcohol and then put into a flame in order to free it from microorganisms. As an added precaution against introducing contamination, these transfers were always made in an inoculation chamber.

Germination of grains

Following their transfer to agar plates, the corn grains were germinated in darkness at, or near, room temperature for a period of 5 to 7 days. Daily examinations were made, without removing the Petri-dish covers, and a record was taken of the germination and number of infected grains. The germination of corn grains, as the result of this treatment, is shown in figure 1. In this case, 8 of 10 grains had germinated. None gave evidence of being contaminated with microorganisms.

![Figure 1. Sterilized corn grains germinated under aseptic conditions.](image-url)
Experimentation

Effect of Varying Time of Treatment

Several experiments were carried out to determine the effect of different lengths of treatment. Two of these experiments will be described. In both cases the Krug variety of yellow dent corn was used. The corn was furnished by Mr. John Trost of the Purdue Agricultural Experiment Station. The disinfecting solutions contained 1 per cent. chlorine. This was checked by titration.

![Graph](https://www.plantphysiol.org)

**Fig. 2.** Effect upon germination and infection of corn grains of varying the length of treatment with sodium hypochlorite solution.

In the first experiment, four lots of 70 grains each were sterilized for 0.5, 1, 3, and 5 hours, respectively. The results of this experiment are shown in figure 2. From this graph it can be seen that increased time of treatment somewhat depressed the germination, and markedly decreased the number of grains which developed infection during the 5-day germination period. The 100 per cent. germination achieved by the grains sterilized for but 0.5 hour fell to 80 per cent. when the sterilization period was lengthened to 5 hours. At the same time, however, an infection of 13 per cent. of the grains which developed with the shortest treatment was reduced to less than 2 per cent. for the longest period.

The second experiment (fig. 3), in general, confirmed the results of the first experiment. Five lots of 100 grains each were sterilized for periods of 1, 2, 3, 4, and 5 hours. Two curves of the graph show that greater duration of treatment over a 5-day period, serves to depress germination ($G$), and greatly to reduce the number of infected grains ($I$). A third curve of the graph shows the effect of the longer periods of treatment in the reduction.
in size of the roots (W). The reduction in the size of the root, attained within a 5-day period of germination, is associated with a slower rate of germination. This was also observed for other than the shortest treatments.

Suction Treatment

It was believed that a more effective sterilization could be obtained, if the disinfecting solution could be made to penetrate the rough loose tissues at the base of the grain. This belief was based upon the observation that the fungi, which produced the infected condition, invariably were found in this basal region.

An experiment was therefore carried out for the purpose of increasing the penetration of the sodium hypochlorite solution, and thereby increasing its effectiveness. Equal quantities of the disinfecting solution were added to two lots of 100 grains each. One of the flasks containing grains and solution was attached to a suction pump for one minute. This resulted in the rapid removal of considerable air in the form of bubbles from the grains. When the suction was released, the evacuated space was filled with solution. The other flask was not connected to the suction pump, and served as a control. Both flasks were shaken 2.5 hours.

The results of this experiment are shown in figure 4. It is evident that while the suction treatment reduced the germination over a 5-day period, as shown by the graph on the left, it also markedly reduced the number of infected grains. Of the 100 grains in each set, 9 grains of the control group became infected; only 1 grain was infected when the suction treatment was applied, as shown by the center graph. On the basis of 100 grains germinating with each treatment, 11.5 per cent. of the controls were infected,
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FIG. 4. Effect of suction injection treatment upon germination and infection of corn grains sterilized with sodium hypochlorite solution. Control (C) without suction treatment; grains (S) with suction treatment.

but only 1.75 per cent. of those exposed to suction treatment were infected, as shown by the graph at the right of the figure.

Summary

1. Increasing the length of time of treatment of corn grains with sodium hypochlorite solution from 0.5 to 5 hours reduced germination somewhat, whereas it greatly decreased the percentage of infected seed.

2. When sterilization with the disinfecting solution for an intermediate period of time was accompanied by a suction injection treatment, there was a marked reduction in the number of infected grains.

Purdue University
Lafayette, Indiana

LITERATURE CITED


