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A METHOD OF GROWING BACTERIOLOGICALLY STERILE POTATO PLANTS

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INTRODUCTION

Studies of different phases of the diseases of the potato (*Solanum tuberosum*) caused by Fusarium species were carried on from 1916 to 1920.

In the Western States the most serious fungous diseases of the potato are those which exist in the soil or develop and attack the plant through underground parts. To define precisely the disease symptoms on the potato caused by Fusaria or other soil fungi has long seemed impossible. The symptoms vary widely, with apparently like causes. A similar type of symptom was usually obtained in inoculation experiments, but it was desirable to obtain greater definition and precision in this respect. The most logical method in the solution of this difficulty appeared to be the development of plants free from organisms of all kinds, a bacteriologically sterile plant growing in sterile soil and in other respects as nearly as possible under field conditions. Given the pure culture of a potato plant, the symptoms resulting from the introduction of pathogenic organisms in pure culture would be free from biological complications. The symptoms so produced might properly be termed "pure." To accomplish this would be of value in the study of many plant diseases.

The attempt to grow sterile potato plants in sterile soil, the apparatus and methods developed to accomplish this end, and the various phases of the experiment and its results are described in this bulletin.

The work was done at the Colorado Potato Experiment Station, Greeley, Colo., at the suggestion of H. A. Edson, of the Office of Vegetable and Forage Diseases. The writer acknowledges his indebtedness to the several men who at various times aided with their skill, strength, and patience in the consummation of the work.

THE APPARATUS

Not all of the apparatus used in this experiment was newly designed. Most of it consisted of commonly available utensils or apparatus altered and improved to meet these special needs. The equipment described by Briggs and Shantz[1] was adapted for use here, and where similarity of tool or method is revealed they have priority to the idea.

A first requirement was that the apparatus should be substantial throughout, to withstand hard and rough usage and neither break nor give way under ordinary stress. The cans filled with soil were heavy; they had to be moved, worked on, and otherwise handled while at a high temperature. No piece of apparatus could be tolerated whose failure to function resulted in physical injury to men, in time lost, or in the undoing of some previous act. A failure in apparatus, as well as in skill, might in a moment invalidate the labor of many previous months. To meet these requirements the apparatus was altered as the need became apparent. It is not intended to give a history of the development of the entire equipment, but only a description of the things ultimately used.

THE CANS AND THEIR COVERS

One hundred cans were used, identical in every essential respect. These were small ash cans of heavy galvanized iron, corrugated throughout the barrel and having a heavy iron rim at the top and bottom. The bottom was slightly lowered in the center. There were two drop handles on opposite sides and two bail eyes directly above the handles and 4 inches from the top of the rim. Before use the cans were carefully inspected for breaks or weak spots in the galvanizing or solder, and these were repaired by soldering. The top joint between the iron rim and the barrel became broken easily, and constant care was necessary to keep these joints perfect.

The cans were 26 inches high and 16 inches in diameter. The weight of water required to fill a can to the brim was 72.7 kilograms (160.27 pounds), indicating that the cubic content of a can was approximately 72,700 cubic centimeters (about 4,436 cubic inches).

The covers fitted the cans tightly. They were molded in form, a narrow shoulder meeting the rim of the can, the cover lapping over

[1] BRIGGS, L. J., and SHANTZ, H. L. THE WATER REQUIREMENT OF PLANTS. I. INVESTIGATIONS IN THE GREAT PLAINS IN 1910 AND 1911. U. S. Dept. Agr., Bur. Plant Indus. Bul. 284, 49 pp., illus. 1913.

it for 1 inch. The crown of the cover rose above the shoulder 1 inch. In their original state they were provided with substantial handles riveted in the center.

The completed cover is illustrated diagrammatically in Figure 1.

The middle part of the handle was cut off, leaving the two upright ends, 1½ inches long, in each end of which a small hole was made. In the very center of the cover, a hole $1\frac{7}{16}$ inches in diameter was made. On an axis at right angles to the axis of the original handle, and on a center 6¼ inches from the center of the cover, a hole 1½ inches in diameter was made. In this hole was soldered a brass tube 2 inches long, extending an equal distance above and below the cover. On the opposite side of the center an irregularly shaped hexagonal opening was cut, two sides being on radii at right angles to each other from the center of the cover, beginning 2⅜ inches from the center and extending for 2½ inches, at which points two other sides began, at right angles to the sides formed along the radii and extending for 2 inches. The other two sides were parallel, the center of the nearest being 1⅝ inches from the center of the cover, the side being 3½ inches long and the center of the opposite side being 4⅞ inches from the center of the cover, this side being $4\frac{7}{16}$ inches long. As these cuts were made with a chisel, accuracy in every cover was not easy and some slight deviations resulted. The pieces of metal which were cut out were reshaped, the edges brushed with a file and refitted to the hole. A lip three-sixteenths inch wide was soldered underneath for the cover to rest upon. An identification number was stamped on each cover and inset piece.

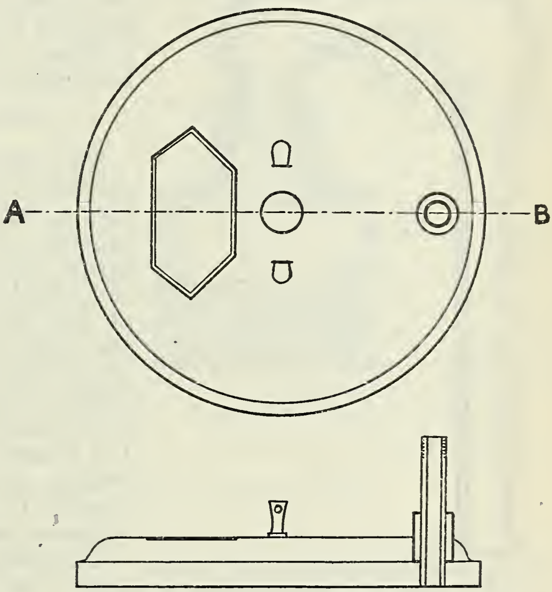


FIG. 1.—Diagrammatic representation of the surface view, and cross section on the line A–B, of a can cover. The irregular hexagonal opening, the hole at the center, and the water pipe inserted in the brass tube are all centered on the line A–B. The upright ends of the remnants of the handle are shown in the cross section

In the brass tube soldered in the round hole made in one edge, a ¾-inch pipe, deeply threaded at one end, was set in plaster of Paris. The inside of the ends of the pipe were carefully reamed to remove any roughness or constriction. The pipe extended through the tube to the plane of the edge of the cover. After the plaster of Paris was well dried it was sealed with a stiff sealing wax.

Figure 1 shows the openings and their arrangement on the cover, all openings being arranged symmetrically on the line A–B. A cross section, made on the line A–B, shows the arrangement of the pipe in the tube and the tube in the cover. In the center the remnants of the handle project upward, with holes in the ends.

The upper (threaded) end of the pipe projecting from the cover was fitted with a vent cap, consisting of a ¾-inch to ⅛-inch reducer, two ⅛-inch elbows, and a ⅛-inch pipe 3 inches long, and connected in this order. A cross section of the vent cap assembly is shown in Figure 2.

The interior of the elbows and the upper two-thirds of the pipe were closely packed with asbestos wool. As already noted, the pipe was deeply threaded. The interior of the cap was painted with oil and graphite, which permitted it to be screwed on fully and tightly and yet allowed its easy removal with freedom from excessive friction or binding after much steaming at indeterminate intervals.

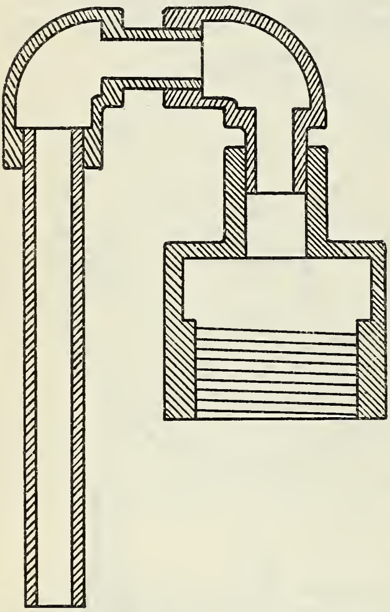


FIG. 2.—Cross section of the vent cap. It consists of a ¾-inch to ⅛-inch reducer, into which fit successively two ⅛-inch elbows and a ⅛-inch pipe 3 inches long and open at the end. The elbows and upper two-thirds of the ⅛-inch pipe are closely packed with asbestos wool

SOIL-STERILIZING KETTLE

The kettle in which the cans of soil were sterilized was a high-pressure process kettle. The ordinary process kettles used in canning factories are of comparatively light construction and endure a pressure of not to exceed 25 pounds to the square inch. Although that used in the experiments was identical in design with the lighter kind, it was of much heavier construction, the hinged and counterpoised lid having an increased number of clamps (11) and the pressure adjustment being 65 pounds.

The kettle rested on beam supports over a large pit and was provided with four heavy lugs at the center, which held it upright. One-half of the kettle was below the floor of the room, leaving a distance of some 3 feet which the cans had to be raised to be put into it. The approximate inside dimensions of the kettle were 4½ feet in diameter by 6 feet high, measuring from the perforated false floor in the bottom. The steam line entered near the bottom at one side. In the center of the convex bottom a drain of 2-inch pipe led off to one side and closed with a valve operated from above by a rod. From the rim at the top another 2-inch pipe led downward, closing with a gate valve.

On the left side there was a steam-pressure gauge and thermometer, the latter being afterwards dispensed with as an unnecessary obstruction. The safety valve in the lid was set to release at 65 pounds pressure. In the upper rim were four ¾-inch holes threaded for pipe. These were all plugged except the extreme left one. This was supplied on the inside with one half of a brass pipe union. The arrangement of the attachments to the kettle appearing above the floor line is shown in Plate 1.

THE BOILER

Steam was generated in a vertical steam boiler of a size rated at 5 horsepower. The boiler had all the customary attachments of pet cocks, gauge, injector, and a safety valve set to release at 100 pounds pressure. The steam was carried directly to the kettle through a 1-inch steam line controlled with a single gate valve. This boiler is shown in Plate 1 behind the kettle.

TRANSPORTATION OF CANS

After filling and before planting the cans were moved many times. For convenience in handling them two pieces of 1-inch pipe 5 feet long with hooks in the middle were used. The hooks were caught in the bail eyes and the can lifted and carried by two men. The hooks were made by welding a ring around the middle of the pipe, the ends being drawn out and bent into hook form. The method of drilling the pipe and passing the straight shank of a hook through it failed, as the holes so weakened the pipes that they bent easily.

At the kettle a half-ton chain block suspended from a movable trolley rolling on an I beam overhead was used. The cans weighed about 260 pounds (118 kilograms) and had to be raised at least 3 feet above the floor and lowered 6 feet into the kettle. Any point in the kettle and for 6 feet in front of it was accessible to the block and trolley. The cans were suspended from the hook of the block by an evener. The evener was made of a crucible-steel tube 16 inches long, with strong, short drop chains and hooks at the ends to attach to the bail eyes, and a ring at the center to suspend it from the block.

The chain block, trolley, and a portion of the I beam appear in Plate 1.

ELECTRICAL-RESISTANCE THERMOMETER

For the purpose of ascertaining the temperature in the interior of the cans and kettle an electrical-resistance thermometer was used. The three wires from the resistance bulb were encased in a lead cable, which terminated in the complementary portion of the pipe union, the other part of which was attached to the rim of the kettle. The resistance bulb was planted in the center of a can, the soil properly packed around it, the free ends of the wires passed through the hole in the rim, and the union connected. The wires were properly attached to the indicator outside. These are shown at the left of the kettle in Plate 1.

ASEPTIC CULTURE HOUSE

When a sterilized can was opened for any purpose this had to be done under aseptic conditions. As the cans were difficult to handle and as they usually remained open for 20 to 30 minutes at a time, a special culture house was provided in which they could be manipulated.

This house consisted of a practically air-tight box, 6 feet in each dimension, and well painted inside and out. A narrow doorway at the front, closed with a sliding door, was the only opening. The glass panes of two small side windows were sealed in. Before the door was a small platform. Two small half-round tracks ran through the center of the room and to the edge of the platform. A small carriage with a swivel top was pushed back and forth along this track. The carriage could be pushed outside of the door, a can deposited on it, the whole pushed inside, and the can rotated as desired.

On each side of the interior were shelves extending the entire length. They were made of thin strips of wood turned edgewise with spaces between. This gave sufficient strength, but allowed water to fall through. Directly overhead was a water sprinkler of the type used in protective systems. The operator, after entering the building with all necessary apparatus, closed the door and turned on the sprinkler, wetting down the walls and precipiting all dust. By standing beneath the sprinkler the operator escaped a wetting. The culture house and the boiler house are shown in Plate 2, B.

THE WEIGHING FRAME AND BALANCE

The cans were weighed frequently to determine the water loss. The frame devised by Briggs and Shantz[2] was used for this purpose. It consisted of four upright pipe posts 8 feet high forming a frame 2½ feet square. At the top, between the midpoints of two connecting pipes, was a shaft with a small gear at the end. An endless chain dropped down to one post, where at a convenient height was a similar gear turned by a hand crank. At the bottom of the two pairs of posts between which the overhead shaft extended were two wooden skids to which the frame was fastened and on which it was moved about.

From the shaft above a short rope loop was dropped, in the sag of which hung a small pulley and hook. By turning the crank the rope was wound around the shaft and the pulley and hook were raised. The endless-chain arrangement was greatly improved by placing a sprocket wheel of large size midway between the two gears and between the vertical parts of the chain. The sprocket wheel merely rotated in place when the crank was turned, kept the chain taut, and prevented it from slipping and doing damage to the balance.

The balance was of special design, of the spring-balance type. Instead of recording from zero to capacity, it began at 50 kilograms. This permitted the use of a more positive spring, better adapted to the heavy weights and the rough usage it received. The 12-inch dial was graduated to half kilograms. At the end of the indicator was a 5 point vernier which permitted the scale to be read easily to 100 grams.

THE WATERING MACHINE

It was necessary to supply the cans with water in order to replace that lost by evaporation and transpiration. The water needed to be supplied in quantity, in a sterile state, and under aseptic conditions. Contamination of the cans might easily have been brought about by an impure water supply, carelessly handled.

To meet this need a machine was devised in which water in quantity could be sterilized and dispensed as needed. It consisted essen-

[2] See literature cited in footnote 1.

tially of a supply tank, an air-pressure tank, and two measuring cylinders, all made portable by being mounted on a substantial hand truck. The machine is shown in Plate 2, A.

The details of the supply and air-pressure tanks with their connections are shown in Figure 3.

The air tank was suspended beneath the truck; the water tank of 66 gallons capacity being fastened above. The air-pressure tank opened to the air through the valve A and to the water tank through the valve B. Above valve B was a small cylinder at G tightly packed with asbestos wool. Above G was a check valve C and also a pipe H entering the convex end of the water tank, bending upward, the open end terminating as near the top as possible. At the point I a pipe led out above to an elbow, on top of which was valve D and at the side of which was a pipe provided with a valve (E) and ex-

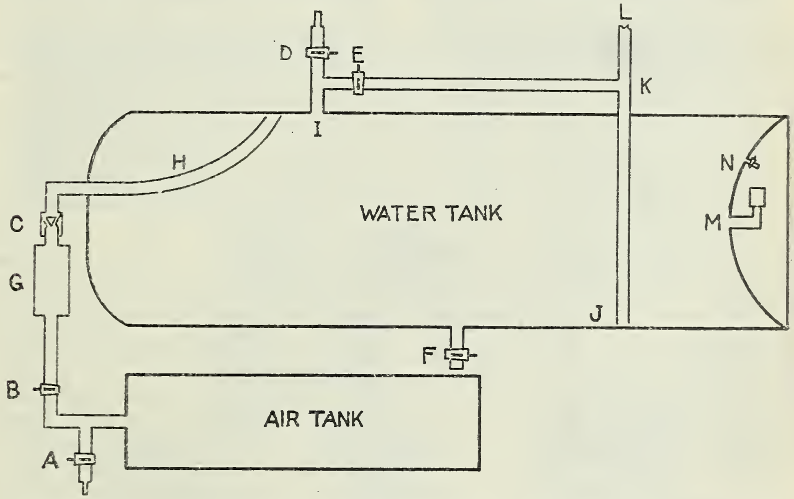


FIG. 3.—Diagrammatic cross section of the tanks and connections of the watering machine (except the cylinders). The high-pressure air tank is reached by way of the valve A. Air escapes through valve B into the cylinder G packed with asbestos wool, through the check valve C and pipe H, to the top of the water tank. Water is drained and steam admitted to the water tank through the valve F. The tank is filled through the valve D and pipe at I. The valve E is used only at time of sterilization, being closed at other times. The water is forced up through the pipe J and out at L. At M is a pressure gauge and at N a pet cock

tending toward the front of the tank. In the bottom of the tank was valve F. In the concave end of the water tank was a pressure gauge M and a pet cock N. A pipe J whose free end was close to the bottom of the tank led upward to the outside and into a tee at K, at which point the pipe from valve E was attached. The upper opening of the tee led into a pipe shown broken at the point L.

This pipe led to the measuring and distributing cylinders, the details of one being shown diagrammatically in cross section in Figure 4.

The pipe L, broken at the bottom, is a continuation of the pipe L of Figure 3. The top of pipe L terminated in a three-way right-angle valve O, which served to direct the water into the pipe P, leading to the right-hand cylinder, identical in every respect with the cylinder R (shown in fig. 4), to which the pipe Q leads.

The cylinder R was made of 4-inch pipe capped at each end and was of slightly more than 2 liters capacity. At the bottom of the cylinder the shut-off valve Y was attached with a close nipple, and below it was a swivel joint Z. From there a flexible metal hose 4 feet long extended, the free end having a kind of metal cuff attached, as will be described below. In the upper end of the cylinder at the point S was a pet cock. In the center a ¾-inch pipe about 6 inches long extended upward and was in reality a valve. Inside of this pipe was a float T, consisting of a thin, conical, turned-brass cap on top of a large piece of sunflower pith. The pith below the cone was covered with lightweight sheet brass, the whole being soldered air-tight. This made a fairly rigid but very light float, which floated in water readily. It was large enough to fill the interior of the pipe yet moved freely without lateral motion. A wire at W, soldered to the cap of the cylinder and across the opening, prevented the float from dropping into the cylinder. At the end of the pipe was the valve seat U, made of a pipe coupling filled with Babbit metal and turned to make a perfect seat for the cone of the float, and having a small hole at the center. With the valve seat U in place, a vertical movement or rise of the float of not more than one-eighth inch effectively closed the opening in the valve seat. The pipe V, above the valve seat, led over to the valve seat of the right-hand cylinder, not shown in the figure.

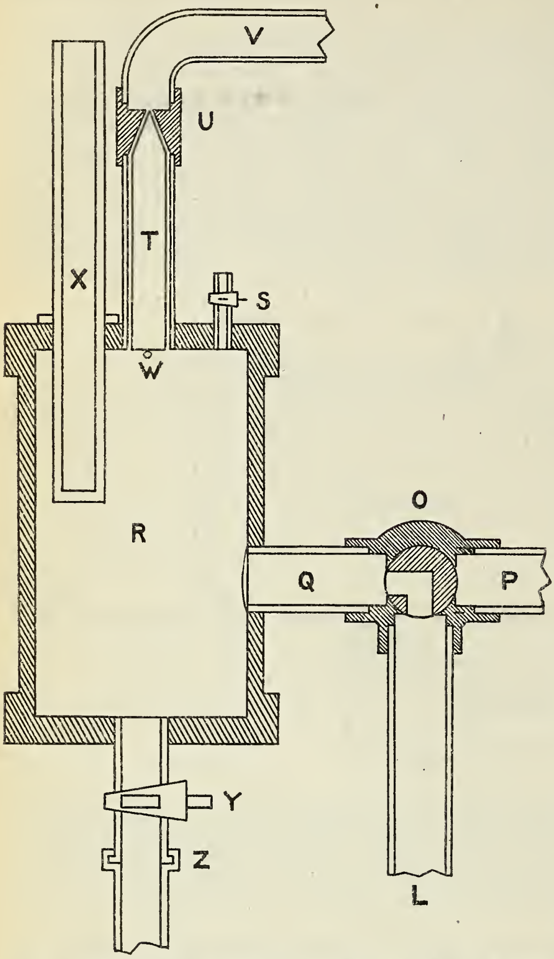
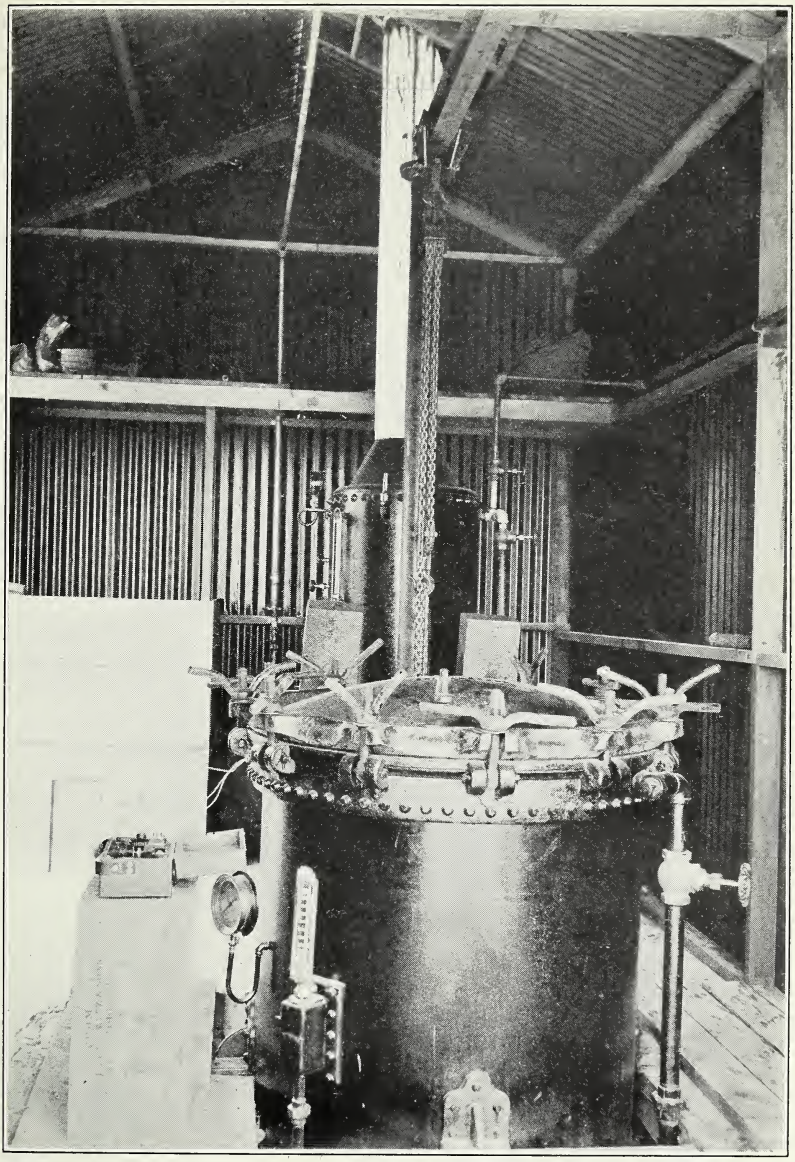
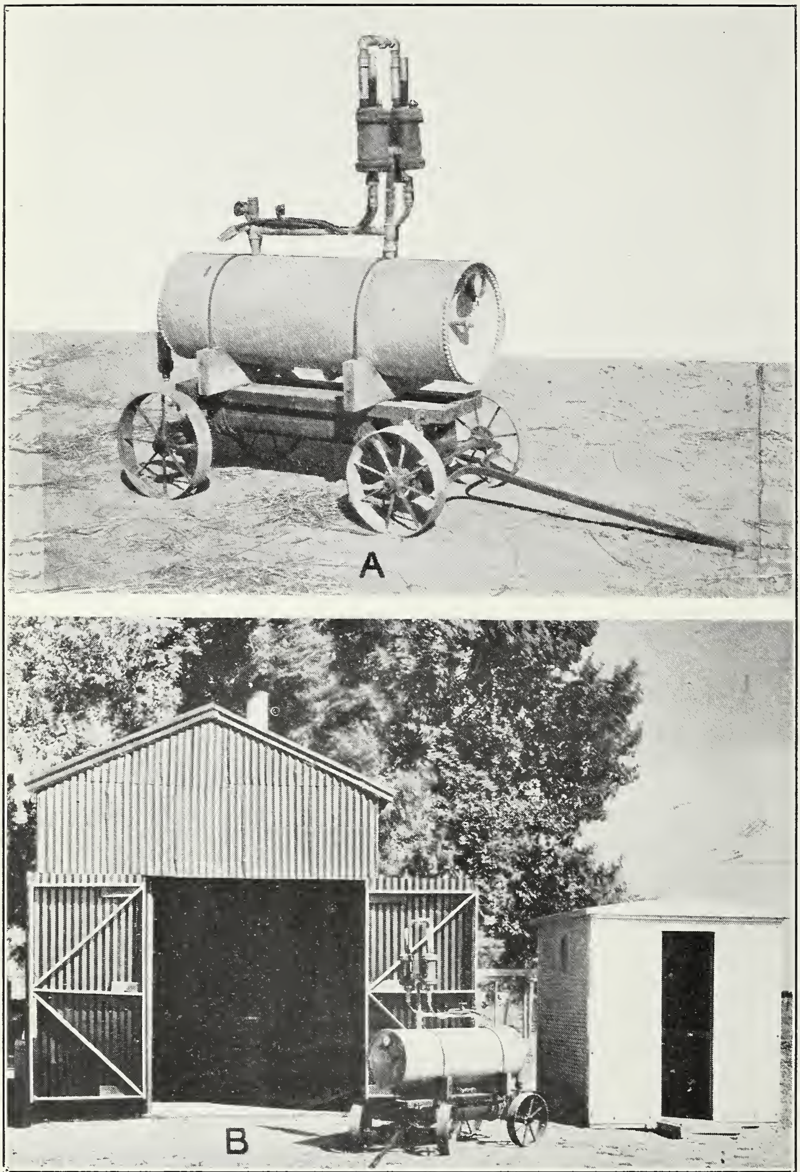


FIG. 4.—Diagrammatic cross section of a measuring cylinder with its connections. The water entering at L is diverted by the valve O through the short pipe Q into the cylinder R, or through the pipe P into a similar cylinder not shown here. In the bottom of R the outlet is closed by the valve Y, beyond which is the swivel Z leading to the metal hose. In the top of the cylinder R is a pet cock S, also a float T, fitting into the seat U when raised by water and prevented from falling into the cylinder by a cross wire at W. A hole through the valve seat U leads into the pipe V, which connects with the other cylinder in reverse order from that shown here. The closed pipe X, sealed with a lock nut, screws in or out of the cylinder to calibrate it to deliver precisely 2 liters of water



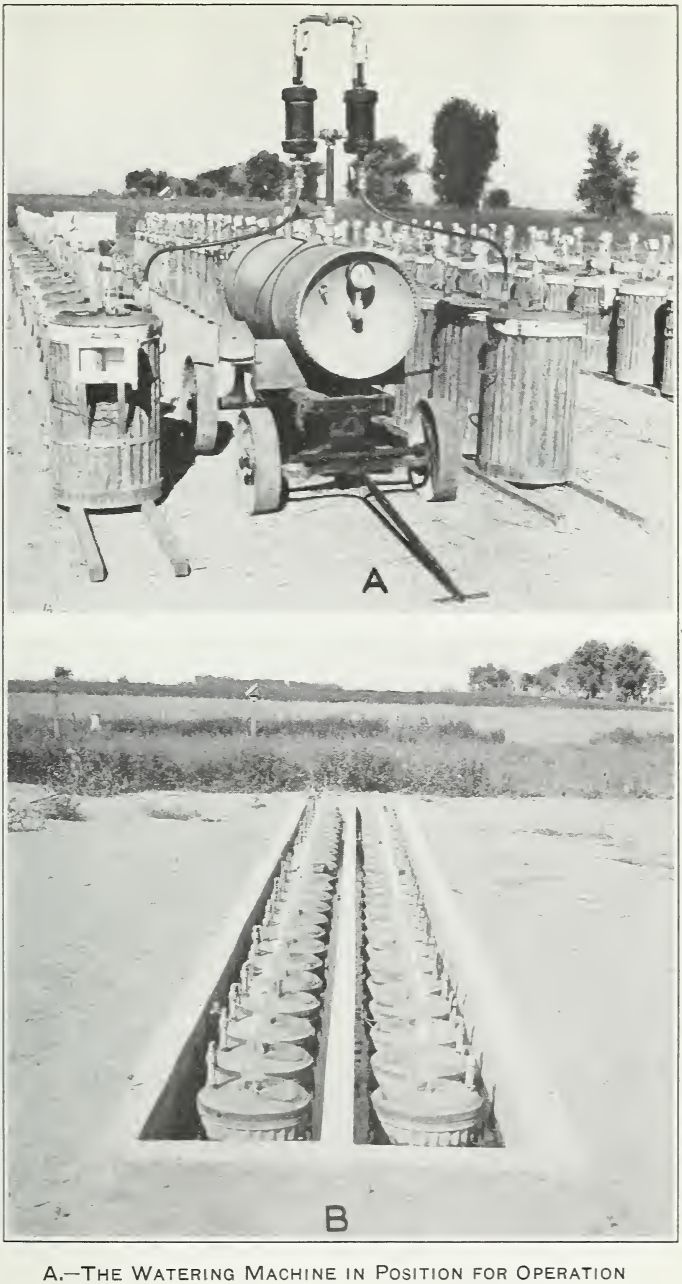
KETTLE FOR STERILIZING SOIL CANS, WITH ATTACHMENTS AND OTHER APPARATUS

The kettle, in the foreground, rests on beam supports, being held by four lugs, one of which appears at the bottom center of the kettle. At the right a waste pipe from the rim leads downward and is controlled by a gate valve, shown here. At the lower left side is a protected thermometer, beyond which is a pressure gauge. On the box is an electrical resistance thermometer, the three wires entering the kettle through the rim. The lid is shown closed with 11 clamps. Immediately behind the kettle appear two iron blocks, which counterpoise the lid. On top of the lid is a safety valve. The lower half of the kettle extends through the floor of the room. Behind the kettle and between the iron blocks is an iron post supporting an overhead I beam, on which is a trolley, from which a chain block hangs. In the background a 5-horsepower steam boiler is seen, showing cocks, gauges, safety valve, smoke pipe, and two steam lines at the rear, the upper one leading outside the building, the other leading downward to the kettle



A.—THE WATERING MACHINE
B.—GENERAL VIEW OF THE BOILER HOUSE AND THE WATERING MACHINE

The culture house is shown at the right



A.—THE WATERING MACHINE IN POSITION FOR OPERATION

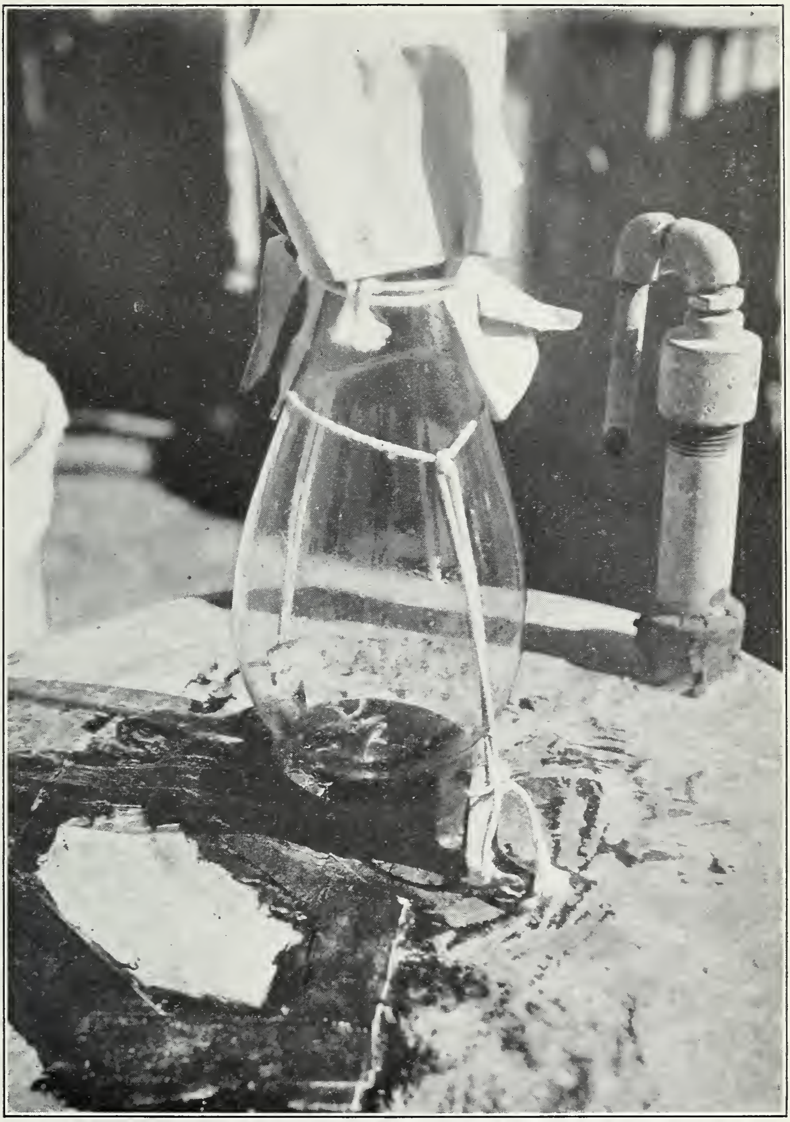
The two rows of cans are arranged with the watering pipe toward the machine and far enough apart to permit opposite cans to be reached by the flexible metal hose at the same time. After being flamed the vent caps are removed and the ends of the metal hose flamed and inserted in the can, the cuff coming over the outside of the pipe. The mittens which cover the end of the hose when no water is being delivered are shown above, on top of the calibrating plungers. This figure also shows the method of attaching a soil thermograph

B.—CONCRETE TROUGHS CONTAINING CANS

In these troughs the soil cans were set in water for the purpose of maintaining a more even temperature. The water could be changed at will

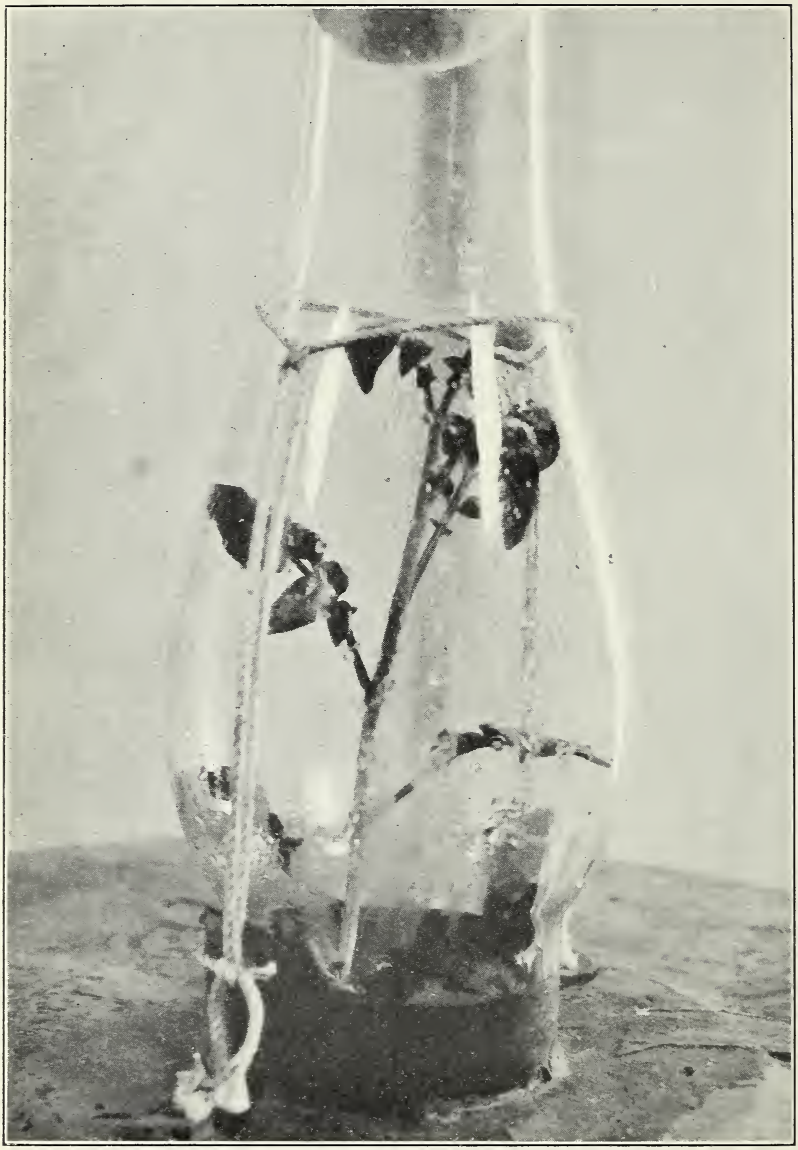
CANS OF SOIL AFTER STERILIZATION

The method of sealing the cans after sterilization and prior to planting is illustrated. The water pipe and vent cap are shown at the back of the can in the foreground, the upright remnants of the handle in the center, and the hexagonal opening and hole at the center of the cover sealed with tape. The joint between the cover and the rim of the can is sealed with tape



COMPLETED COVER EQUIPMENT FOR THE GROWTH OF THE PLANT

At the right rear is the water pipe with vent cap In the center the lamp chimney, tied with cord to the remnants of the handle, covers the hole at the center through which a sterile potato plant is appearing. The cotton plug of the chimney is covered with a paper cap, tied on. In the left foreground the hexagonal opening, sealed with tape and varnish, is to be seen



STERILE POTATO PLANT, SHOWING MAXIMUM GROWTH

At the left of the center is shown the calibrating plunger X. This consisted of a ¾-inch threaded pipe, closed at the end within the cylinder and fastened outside with a lock nut. The plunger was screwed in or out as was necessary to cause the cylinder to deliver precisely 2 liters of water in operation.

The flexible metal hose or pipe, mentioned as coming from the swivel Z at the bottom of the cylinder, was the right external diameter to fit inside of the ¾-inch pipe in the can cover, shown in Figure 1. At a point 2¼ inches from the end of the pipe a sheet-iron cuff was soldered, which was large enough to fit closely over the outside of the ¾-inch pipe of the can cover, and extending over it on the outside as far as the flexible pipe did on the inside. When the flexible pipes were not in use the ends were encased in a close-fitting metal box or mitten, drawn tight with thumbscrews.

Plate 3, A, shows the method of attaching the flexible metal pipes of the watering machine to the can covers.

MINOR EQUIPMENT

SOIL THERMOMETER

As shown in Plate 3, A, recording thermometers were supplied to some cans. These were the common type of soil thermograph, recording on the paper of a cylinder rotating once in a week.

WATER TROUGH

In Plate 3, B, the cans are shown submerged in water for the purpose of lowering the temperature.

The trough was built of concrete. The interior dimensions were 42 feet long, 2 feet wide, and 2½ feet deep. Each side accommodated 25 cans. The water was taken from an irrigation ditch 125 feet away and was pumped in and out at will by a centrifugal pump driven by an electric motor. A single trough with cans in place could be filled with water in 20 minutes.

LAMP CHIMNEY

The lamp chimney which appears in Plates 3, 5, and 6 was 10 inches high and made of heavy clear glass. The top was straight and smooth, and the top opening was filled with a tight cotton plug having a cork in the center to give it form.

GASOLINE BLOWTORCH

Two hand gasoline blowtorches of the kind used by plumbers were in constant use. The gasoline was vaporized and ejected under pressure into a Bunson-type burner and delivered a flame of great heat intensity. They were not fully reliable, so two were always in readiness, even though only one was needed. Steam, wind, or lack of air would sometimes suddenly extinguish one at a critical time, so a second one was always lighted and ready for such an emergency.

There were some other minor pieces of apparatus used in the experiment, but not of enough importance to be recorded. Their use depended on the wish of the operator and not on any imperative demand of the experiment.

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CHARACTER OF SOIL

The soil used throughout the experiment was taken from the fields of the Colorado Potato Experiment Station. This soil is classified by the Bureau of Soils, United States Department of Agriculture, as a Billings loam. It contains some sand and enough clay to cause it to adhere strongly in large lumps when slowly dried. It is free from gravel except for traces of fine gravel found irregularly. The color varies with the quantity of moisture present from brown to dark gray. The soil below the customary depth of plowing is of a lighter color and has more nearly the consistency of clay. The soil used for the experimental work was taken from the top 8 inches. It was free from stones and gravel and contained some humus. Several hundred cubic feet of this soil was taken from the field and shoveled over from pile to pile until it was thoroughly and uniformly mixed. No other type of soil was used.

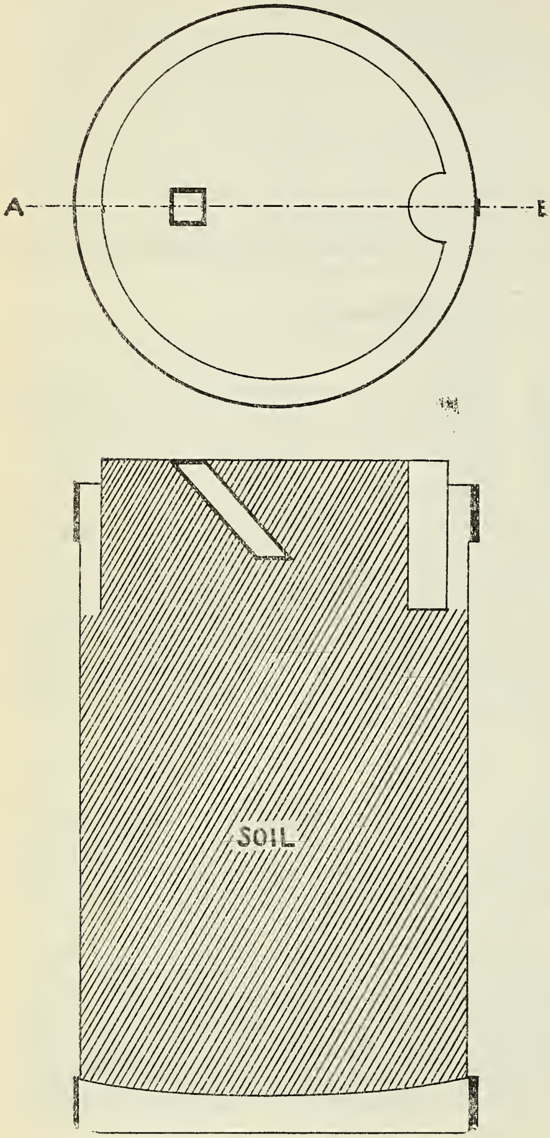

FIG. 5.—Diagrammatic representation of a top view of a filled soil can, showing the water shield and wooden block in place; also a vertical cross section of the can on the line A–B

METHODS AND TECHNIQUE

The methods and technique were developed and improved with time and experience. The method of conducting the experiment can be explained, but the actual technique of manipulation can be but poorly described. Many failures led to changes in the methods, and greater skill improved the technique. It is intended to discuss here only the system ultimately used.

FILLING THE CANS WITH SOIL

The cans were filled with approximately 115 kilograms (253.53 pounds) of soil. A lesser quantity would collapse somewhat under steaming or watering, and a greater weight would bulge in heating or fail to transmit the water properly. The soil was added a little at a time and tamped with a small post or pointed stick. Within a few inches of the top a galvanized-iron water shield was put in place. This shield was made of a strip 6 inches wide and long enough to make a hoop 14 inches in diameter. At the side adjacent to the joint of the top rim of the can a semicircular fold was made to fit around the pipe extending through the cover. The shield was jointed loosely by folding the end edges back and catching one into the other. The shield was raised 1 inch above the rim, which brought the soil against the cover when it was in place. A block of wood 4½ inches long by 1½ inches square, the ends cut at an angle of 30°, was put into the soil so that the lower end came directly under the center hole in the cover and the upper end at the edge of the large hexagonal opening. No soil remained outside the water shield, but inside it was well packed and left no space between the cover and soil when the cover was in place. After these details were completed a ⅜-inch iron rod was inserted anywhere near the rim of the can, pushed to the bottom, and withdrawn.

Figure 5 shows the diagram of the top view of a filled can, also a cross section of it on the line A–B. When the cover is placed on the can, the line A–B of the cover shown in Figure 1 coincides with the line A–B of Figure 5.

FILLING THE KETTLE

After the cans were filled with soil they were ready to be sterilized. The kettle held six cans and covers and the accessories used in sterilization. Three cans were placed in the bottom, the cans so turned that the bail eyes were immediately accessible for attaching the hoisting device. The covers, turned on edge, were deposited beside the cans. Over each of the three bottom cans a large piece of sheet iron was placed at an angle to carry off the water of condensation which dripped from above. After the bottom cans were arranged a tripod made of iron pipe was put in, and on this the three top cans rested, care being taken that no can in the upper tier rested against another can. The covers were conveniently placed edgewise beside the cans. The thermometer bulb was inserted in the soil of one can, the wires passed through the rim, and the union joined. The lid was clamped down and the 11 clamps strongly tightened, using a short pipe for a lever. The contents of the kettle were now ready for sterilization.

STERILIZATION

The pet cocks and all escape valves on the kettle were opened wide and the steam turned on slowly. As the air began to be displaced more steam was admitted, and after a few minutes the valves were closed and finally the pet cocks also. The steam could then be admitted as fast as the pressure in the boiler would allow. The boiler pressure was kept at the highest point possible, in order to have the steam carry the greatest degree of heat. Low-pressure steam quickly condensed on striking the cold kettle and cans.

When the kettle was operated for the first time during the day it was brought up to the required pressure of 65 pounds very slowly. The valves were opened several times to blow off the condensation water and carry out the air. As the pressure in the kettle gradually increased and reached 65 pounds, the pressure in the boiler was dropped to that point, as there was no pressure-reducing valve in the system. The pressure and temperature in the kettle depended directly on the pressure of steam in the boiler, and it was only by good boiler management that the pressure remained even and at the maximum.

REMOVING AND SEALING THE CANS

At the termination of the necessary period of steaming, the valve in the steam line from the boiler was closed and the pressure in the kettle released as quickly as safety would permit. It was desired to remove the cans while they were hot. Usually three persons took part in this operation, each one having specific tasks to perform. Efficient speed was highly desirable. The lid of the kettle was loosened as the pressure diminished, so that as soon as the pressure gauge fell the last time the lid was thrown back. The volume of rising water vapor and heat carried the dust away from the kettle, though the building was usually sprayed previously from an overhead sprinkler to keep down any dust. The thermometer cable and bulb were removed. The surfaces of the cans were quickly and thoroughly flamed with a gasoline blowtorch and the covers put on. The three cans in the top layer were lifted out and the lower three cans were flamed and covered. As each can was lifted out the rim was painted with sterile glue and a piece of sterilized heavy canvas cloth was wound around it, closing the joint between the cover and the can. The hole in the center of the cover was closed with a double piece of adhesive tape, as was the hexagonal opening. All of these openings were thoroughly flamed with the gasoline torch several times in the interval between their removal from the kettle and sealing.

The act of sealing had to be accurately and expertly done (pl. 4), as a defect would inevitably have resulted in contamination. The tape had to remain in place for an indefinite time and endure much hard usage. As soon as the six cans had been prepared for sealing and none of them needed further immediate care, the canvas strip and adhesive tape joints were painted with a good quality of spar varnish and repainted several times as soon as the previous coat dried.

The cans were then set out of doors, to remain sealed until they were ready to be planted. The kettle was loaded again and the process repeated. By efficient handling, three persons could remove and seal the six cans taken from the kettle in about 15 minutes.

PREPARATION OF TAPES, GLUE, VARNISH, ETC.

The strips of canvas cloth 2 inches wide which were used around the covers were torn to the right dimensions. They were well wrapped in paper and sterilized by steam. An adhesive tape 2 inches wide was also used around the cans, and this was usually sterile as received from the manufacturer. It was treated further by pasteurizing on three successive days. The tape used to seal the openings in the cover was treated in the same manner and was uniformly sterile.

The glue and varnish could not be subjected to steam sterilization, as the volatile parts would have been boiled away. They were pasteurized, and testing discovered no organisms in them. The brushes used were sterilized by steam and varnish.

No contamination of the cans could be traced to the tapes, adhesives, or varnishes used in sealing the cans.

The lamp chimneys were prepared with cotton plugs in the upper ends, tied in strong individual paper sacks, and sterilized at 20 to 30 pounds pressure in the kettle for half an hour.

The tapes, utensils, and other pieces of apparatus or supplies which were sterilized in quantities were sterilized in the kettle.

PREPARATION OF CULTURE MEDIA

A potato agar was much used in testing the potato seed pieces for sterility. It was made by steaming sliced peeled potatoes in water, in the proportion of 200 grams of potatoes to 1 liter of water, decanting the liquor, restoring it to volume, and adding 1 per cent powdered agar. The medium was again steamed for an hour and sterilized at a pressure of 15 pounds for 20 minutes. This agar was usually made in 10-gallon quantities, the steaming and sterilizing being done in the kettle.

Other media used were made under laboratory conditions.

PREPARATION OF POTATO SEED PIECES

The true seed of any plant can usually be sterilized with ease and safety. This is not possible with the potato seed piece. Attempted sterilization at the surface with mercuric chloride or other solutions never resulted in complete sterilization. A number of methods, solutions, and chemicals were tried, to no avail. Upon planting the seed piece on potato agar, bacteria and mold developed.

The only successful method consisted in paring away the surface of the tuber, leaving the raw sterile tissue uncontaminated. The work was done in a closed culture room. The potatoes were thoroughly scrubbed with a stiff brush and treated for 1 hour in 1-to-1,000 mercuric-chloride solution. A single eye was selected as the center of operations. The tuber was firmly impaled on a common fork, and the paring process begun. The knives were flamed before using and only one stroke of the knife was made before flaming it. A knife could be used for only five or six strokes, as the potato juice burned on the blade in the flaming and made it impossible to use it after a few strokes. As much tuber flesh as possible was left with the selected eye, the remainder being cut away toward the fork. The piece was finally cut across the eye. This left a square block of potato on the fork with an eye in the center of the upper side. Then a second round was made, cutting away a thin slice on each side. The top side was cut again, a very thin slice being cut away in the hope of leaving the germ of the eye. Finally nothing remained to be done but to cut off the seed piece above the fork and drop it into a container.

The containers were pint preserving jars with screw tops. An inch or more of potato agar was in the bottom of each jar. The mouth was plugged with cotton. The jars were sterile. The agar was softened by heat so that the potato seed piece sank into it. Usually a few cubic centimeters of potato agar was dashed over the eye of the seed piece from a separate tube. The eyes were always turned upward, so that they could be examined. Any contamination which existed on the seed usually developed on the agar. There were many contaminations, caused by the organisms present in the deep folds of the epidermis about the eye.

In another large number of cases where no contamination developed the eye had been so pared away that it failed to germinate. Both the contaminated and the nongerminating pieces were discarded. A period of two weeks to a month was required to determine finally whether a seed piece was going to germinate properly and was free from foreign organisms. The work of preparing the seed pieces was done in the culture house. The sterile and germinating seed pieces were planted in the cans in soil which had been tested and found to be sterile.

PLANTING THE SEED PIECES

A can from which a preliminary test sample of soil had been taken through the water pipe in the cover and plated on soil-decoction agar and found to be sterile was taken into the culture house. There were also taken two or more jars containing sterile germinating seed pieces, the necessary tools, sterile tapes, glue, varnish, water, gasoline torches, lamp chimneys, and other necessities. The door of the house was closed, the interior sprayed, and the torches lighted. The entire cover and the upper part of the can were then given a drastic flaming with a gasoline torch.

The hexagonal opening of the cover was first uncovered and a sample of soil taken in a sterile test tube. The wooden block shown in Figure 5 was drawn out and a sterile seed piece was taken from a jar and pushed down the hole left by the removal of the block. Care was taken always to arrange the seed piece so that the one bud was in an upright position and approximately under the hole in the center of the cover. Soil drawn away from beneath the hole in the center of the cover was pressed down about the seed piece, smoothed over, and well flamed with a blowtorch. The metal cover for the hexagonal opening was flamed, returned to place, and sealed again after many flamings. The opening at the center was now uncovered and well flamed.

A lamp chimney was taken from a sack, the lower end dipped in a pail of sterile waterproof glue to the depth of an inch or more, and immediately set on the cover. The glue drained to the cover and effectually sealed the opening between the cover and the chimney. A very strong cotton cord was looped about the chimney above the bulge and tied as tightly as possible to the remnants of the cover handle. The details of this arrangement may be observed in Plate 5.

This completed the planting, and the can was ready to be placed in position out of doors for the summer.

When a thermograph was to be attached to a can it was done at the time of planting. The bulb and a sufficient length of wire were immersed in a 10 per cent solution of formalin for two hours and the bulb placed in the soil at the center of the can. The wire emerged at one corner of the hexagonal opening and was well flamed before being sealed in place. The joint was made tight with sterile tape, glue, and varnish, and no contamination was ever observed from the introduction of the thermograph or its presence. The wire was well secured on the outside, so that there was no motion which would break the seal.

PREPARATION OF STERILE WATER

After being planted the cans were set in the open and left for the seed piece to sprout and for the plant to develop. During this interval the cans steadily lost weight, sometimes in fairly large amounts. As they were thoroughly sealed except for the devious passage through the cotton plug of the chimney and the vent of the cap on the water pipe, the only possible loss must have been in the form of water vapor representing a direct loss of moisture from the soil. It was necessary to restore this moisture from time to time.

Before water could be introduced into the watering machine, however, the machine itself had to be rendered perfectly sterile. This was done by attaching a steam line at the valve F (fig. 3) and forcing live steam under a pressure of 30 pounds through all parts of the machine for half an hour. This included all pipes, valves, cylinders and their parts, and the flexible pipes. As some water of condensation would accumulate in the bottom of the water tank, the valve E (fig. 3) was opened in sterilizing the cylinders, in order to use steam free from water. No greater pressure than 30 pounds could be used, lest the brass-covered pith float T (fig. 4) should collapse.

After the kettle and its parts were sterile, the valve E (fig. 3) and valve O (fig. 4) were closed. A hose was attached at the open end above valve D and clean water allowed to flow into the tank until it reached the level of the pet cock N. The water hose was removed and the steam again supplied through the valve F, this time under high pressure. The water soon reached the boiling point, and the tank was boiled by live steam at a pressure of not less than 50 pounds, as shown by the gauge M, for two hours. The acquired water of condensation completely filled the tank. The machine and its contained water were now sterile. All valves were tightly closed, the steam line disconnected, and the machine allowed to cool overnight. It was then ready for use.

SUPPLYING THE CANS WITH STERILE WATER

The operation of watering the cans, though simple, was attended with many precautions. The air tank of the machine (fig. 3) was filled with air under considerable pressure. The cylinder G filled with asbestos wool, the check valve C above it, and the pipe into the tank were flamed with a gasoline torch until red hot. The torch was usually trained on the cylinder G for an hour or more.

The air was slowly admitted through the valve B and the pressure in the water tank maintained at about 15 pounds, as shown on the gauge M. The air, leaving the pipe H, forced the water up through the pipe J to the valve O of Figure 4. The vent cap and pipe of the can cover, shown in diagram in Figures 1 and 2 and also in Plates 4 and 5, were thoroughly flamed with the gasoline torch. The cap was taken off and the opening flamed. The end of the flexible pipe of the machine with the cuff attached and covered by the mitten was thoroughly flamed. The mitten was taken off and the opening flamed before it was inserted in the water pipe of the cover. The valve Y remained closed in the left-hand cylinder and was opened in the right-hand cylinder. The valve O admitted the water from the pipe L into the pipe Q and into the cylinder R. As the water rose in the cylinder the contained air was forced through the valve opening at U through the pipe V into the right-hand cylinder. When the water reached the float T it rose, closing the opening at U, thus stopping the flow of water into the cylinder. The valve O was then turned to direct the water into the right-hand cylinder; the valve Y was opened immediately after, and the water rushing into the right-hand cylinder forced the air back again into the left-hand cylinder, driving the water through the valve Y and the flexible pipe into the can. This operation was performed as fast as the pipes and caps of the cans and the ends of the flexible pipes could be flamed and changed, or as long as water remained in the tank.

Plate 3, A, shows the watering machine in position to irrigate two cans. When the cans were submerged in the troughs, as shown in Plate 3, B, they were raised by the weighing frame and irrigated one at a time.

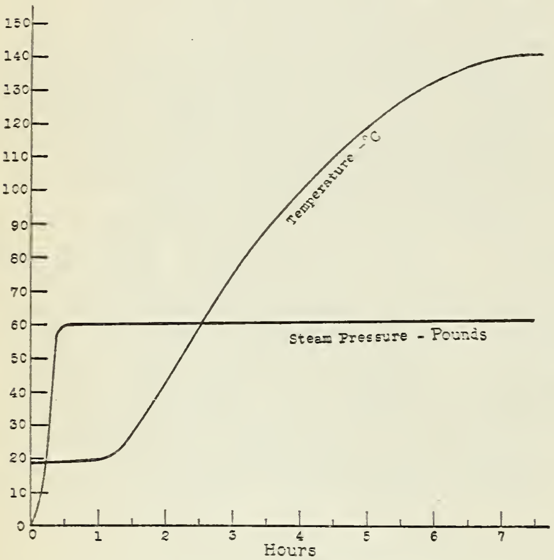

Fig. 6.—Diagrammatic curves of the steam pressure in the kettle and the temperature in the interior of a can of soil in a representative sterilization. In reading the steam-pressure curve the axis of ordinates indicates pounds; in reading the temperature curve it indicates degrees centigrade; and the axis of abscissas indicates time in hours for both curves

WEIGHING THE CANS

In weighing, the balance was hung from the pulley in the rope loop on the overhead shaft of the weighing frame, and an evener similar to the one used at the kettle but with longer side chains, was used to attach the balance to the cans. By turning the crank and winding the rope about the shaft the weight of the can fell on the balance, and when the can was free from the ground the dial reading gave the weight of the can plus the weight of the evener. The variation in the contents of the can could be determined by referring to a tabulated list.

TESTING THE SOIL AND WATER

The samples of soil taken from the cans and the samples of water taken from the watering machine were tested bacteriologically for sterility. The samples were collected in sterile test tubes. A soil-decoction agar, made by extracting 200 grams of soil with 1,000 cubic centimeters of boiling water and adding 1 per cent of agar, was used in the soil test, as well as potato agar. Small quantities of soil taken on the end of a sterile spatula were introduced into the tube of melted agar, the tubes were rolled between the fingers, and the agar was poured into Petri dishes, any resulting growth indicating a contaminated can.

In the water test 2 cubic centimeters of water was added to a dextrose-broth tube with a small vial inverted in it to form a fermentation tube. Dextrose-agar plates to which a few cubic centimeters of water had been added were also poured. When no growth appeared it indicated sterility.

THE EXPERIMENT IN OPERATION

REGARDING SOIL STERILIZATION

In the operation of the kettle the success of the work depended in part on the rapidity with which the steam could be admitted and in part on the initial temperature of the cans. As the work of sterilization was frequently done in the early spring, the cans were sometimes frozen, or at least thoroughly chilled. Cold cans condensed a great deal of steam, making boiler management difficult, tending to fill the soil in the cans with excess water, and in a few cases to puddle them. The cans needed to be warmed as much as possible. So the kettle was usually started in the afternoon and after being unloaded was filled again, the cans warming considerably over night. On starting in the morning the difficulty resulting from condensation was thus partially avoided. During the day the sun warmed the cans which were to be used next. Usually two lots were run in one day, the time commonly required for a lot of six being about nine hours. Occasionally the kettle was operated continuously. The temperature in the interior of a can was always recorded in each lot. Whether the can was in the lower or upper tier in the kettle seemed to make no difference in the amount of heat received, as indicated by many readings. Figure 6 shows the curves of temperature at the center of a can and the steam pressure in the kettle for a representative sterilization.

Table 1 gives the temperature of live steam under pressure, in the range of pressures under which the kettle operated.

TABLE 1.—*Temperature in degrees centigrade and Fahrenheit of live steam under pressure of 50 to 70 pounds*

Pressure to the square inch	Temperature		Pressure to the square inch	Temperature	
Pounds	°*C*	°*F*	*Pounds*	°*C*	°*F*
50	139.5	283.2	65	149.4	301.3
55	143	289.3	70	152.5	306.4
60	146.5	295.6			

The kettle was usually operated at a pressure of 60 pounds but frequently ran to 65 and 70 pounds.

The air in the soil was slow to leave. Had a vent been provided at the bottom of the can, this would have assisted in the operation. However, as such a vent or cock would offer opportunity for contamination and make handling the cans difficult, it was omitted. Instead, an iron rod was pushed down to the bottom of the can, leaving a small round passage which assisted in the escape of the air.

The temperature to which the cans were brought and the time of exposure were no more than enough to cause sterility. Peripheral areas were sterile, but the center sometimes was not. Usually an exposure of a can to 65 pounds of steam for eight hours resulted in sterility. In a few cans sterilized at 60 pounds Pyronema developed. Bacteria were sometimes found. Fungi which developed usually grew luxuriantly and could be detected when the cans were opened. When a can was once found to be contaminated the contents were discarded, as no success was attained in resterilizing contaminated soil.

SOURCES OF CONTAMINATION

The sources of contamination of the cans were many and varied. In addition to the risk of nonsterility due to faulty sterilization, there was the chance of introducing foreign organisms with the water, also with the seed piece. An imperfect joint or seal would let in bacteria or spores under the stimulus of wind, rain, or changing temperature. It was only by the greatest care and drastic application of the gasoline torch that contamination was prevented. The elbows and small pipe of the vent cap (fig. 2) were filled with asbestos wool to exclude organisms. These were heated in place with the gasoline torch, to kill any possible entering contamination as well as to sterilize the thread joint of the top of the pipe.

The top of the lamp chimney, plugged with cotton, was covered with a heavy paper cap, such as appears in Plate 5, to ward off rain, dust, hail, or curious visitors.

The joint between the cover and the can offered considerable opportunity for contamination. The top rim of the can closed with a lap joint, which left an awkward opening under the cover. This was closed with iron cement and solder. The circumference of the cover was larger than the circumference of the rim of the can, so that in drawing a tape or strip of cloth around the joint the lower half was not tightly stretched and might be loose and unattached. Torn pieces of canvas well attached with waterproof glue and drawn almost to the point of breaking made excellent joints. Adhesive tape could not withstand the drawing pressure that canvas stood. In addition, the tape seemed to weaken on exposure to the sun, and the rivet heads in the rim would eventually burst through. By putting on three rounds of tape, gluing and stretching each one on, and tying the whole with a strong cord, a very satisfactory joint was made, and through this no contamination could ordinarily pass.

In spite of all precautions, however, the loss of cans from contamination was considerable. The origin of the trouble could not always be ascertained. Constant application of the torch and the varnish brush were imperative, together with rigid care in handling at all stages of operation.

LOSS OF SEED PIECES

Different varieties of potatoes were used for seed purposes. The Early Ohio was found to be the most satisfactory. If the eyes could be germinated before paring away for sterility, this imparted a greater vigor to the part remaining. The eye of the potato contains folds and crevices impossible to clean by artificial means. They all had to be cut away. The last cut across the eye came close to the remainder of the germinative tissue and in more than half of the cases took all of it. These pieces were useless. Of the others, some would develop contamination at the eye, or molds would appear on them and cause them to be discarded. Where they were of seeming purity and germinating in a degree, it was obvious that they were so weakened by the removal of bud tissue that growth would be problematical. Generally not more than 5 per cent of the seed pieces prepared were satisfactory enough to be used. As each one took much time for preparation, a very considerable amount of time was devoted to this phase of work alone.

EFFECT OF SOIL STERILIZATION

The effect of sterilization on the soil was left undetermined. That was a subject entirely outside the scope of the experiment. That soils are altered by steaming is obvious, and there is a large literature on this subject. It was observed that potato plants grew but poorly in sterilized soil, even when the seed piece was of full vigor. What the act of sterilization contributed to the failure of the experiment is not known, but apparently it was an important factor.

EFFECT OF TEMPERATURE

By the time the cans could be planted it was nearly summer. The cans were set in rows of 25 each in the open, unshaded from the sun. The temperature in the cans under these circumstances rose during the day and fell at night. Because of the changing temperature the air pressure inside of the can had to be easily adjusted to the outside pressure without breaking a seal, and to effect this the vent caps were supplied to each can.

It frequently happened that the temperature ranged through 45° F. during a 24-hour period and seldom through less than 25°. The average maximum was about 90° F. and the mean during the summer was 75°, falling as the season advanced. The potato does its best under cool temperatures, and being planted in cans under trying conditions, it showed no exception to the rule. A few yellow, sickly plants appeared, which ceased to grow in a few days and later died. Some of them were contaminated, but the sterile ones appeared to be killed by the excessive heat.

To control the temperature better the troughs shown in Plate 3, B, were constructed and the water level raised to the highest possible point. This had a marked effect on the temperature range in the cans. The average maximum fell to 80° F. and the daily range to about 15°. The average mean was about the same as with the cans fully exposed and fell in the same way, as the season advanced. The potato plants which grew were of improved vigor and appearance and developed in a more normal manner. Had it been possible to maintain a constant temperature favorable to the growth of the potato, it seems probable that plants suitable for inoculation purposes might have been developed, but with the available equipment this could not be done.

KEEPING THE WEIGHT OF CANS UNIFORM

The cans had to be weighed with care and under known conditions. It was intended to maintain a soil-moisture content of approximately 18 per cent. When the cans lost 2 liters in weight this was restored by the addition of water. These calculations were easily made and the uniform weight maintained.

A very considerable and confusing error would have been introduced by failure to consider the temperature of the air at the time of weighing. The spring of the balance was markedly affected by changes in air temperature, often to the extent of 2 kilograms or more. It became necessary to choose a time of day when the sun did not strike the balance and when the temperature was comparatively low. Screening the balance was tried, but without material success. In order to have a temperature which could usually be attained throughout the growing season, 60° F. was selected as the correct air temperature for the operation of the balance. The weighings were usually made in the morning if the eastern sky was hazy or there was other shade. Occasionally weighings could be made during a cloudy day or after sundown. It was impossible to weigh during the day in full sun.

In weighing, the balance was always tested against a known dead weight, and if the correction necessary was small, the weighings were made and corrected. The cans were not weighed every day, so that a time could be selected without any experimental injury because of postponement.

GROWTH OF PLANTS

The growth of the plants was feeble, even under what appeared to be the optimum conditions. At no time was there anything approaching a normal rate of growth, the plants growing usually only a very few inches during the entire season. On some plants the leaves were yellow and small and, although showing no signs of contamination or disease, were obviously devitalized. The lack of vitality appeared to be caused in part by the soil, the humus of which had been destroyed by the steam. What toxic substances were present in the soil is not known. Also, the plants were definitely hurt by the process of paring, which took away the best part of the eye. From this loss they seemed never to recover. Add to these destructive influences the exposure to unfavorable temperatures, and it is evident that the plant growth must have been weak indeed. Plate 5 shows a small sterile plant emerging from the hole in the cover into the chimney. Plate 6 shows a plant as large as any obtained, but even this developed no special sturdiness.

That these plants were sterile is attested by the sterility of fragments of leaves and roots planted in potato and beef agar. The potato seed piece practically disappeared, but no contamination of it was detected where the leaves and roots were sterile.

INOCULATION

No plants were obtained which could be used for inoculation with Fusaria. This was to have been attempted through the hexagonal opening in the cover. It was expected also that the plants would attain a size sufficient to permit the removal of the chimney from around them and the sealing of the plant in the hole of the cover. The methods and materials were perfected for sealing this joint but were never used in actual practice.

RESULTS

The immediate experimental aim to grow and maintain entire potato plants in a sterile condition during the span of a normal season was achieved. Plants were produced from sterile seed pieces and grown with their roots in sterile soil and the stems and foliage in sterile air. The ultimate object, to add to the known facts regarding the pathology of Fusarium on potato plants, was not achieved. The plants obtained were abnormal, feeble, and lacking in character, so as to be unfit for use as experimental host material.

DISCUSSION

Of the many factors which contributed to the enfeebled condition of the plants produced in the experiments discussed in this article, no single one can be regarded as primary. The principal obstacles appear to have been the weakened and devitalized seed, the poisonous soil, the extremely artificial and unfavorable environment, and the ever-present menace of contamination combated only by drastic means. The potato is a particularly difficult plant to use in such an experiment. If a true seed could have been employed to start the plants, the experimental labor would have been greatly reduced. The lack of uniformity in potato seedlings and the usual weakness of such plants make them unsuited for experimental use in inoculation tests. A growing season long enough to give time for the plants from devitalized seed to recuperate and a regulated temperature could both be attained by artificial means, but there are other environmental factors which are less readily altered and controlled and which are imperfectly understood. The chemical and physical changes in soil which result from steam sterilization at high pressure materially influence fertility, even when every opportunity is offered for the reestablishment of a normal soil flora and fauna.

The technique employed in this work maintained a sterile soil throughout the experimental period and permitted the addition of water as needed without contamination. The methods used might be applicable in a number of experimental problems.

CONCLUSION

A method is described whereby bacteriologically sterile potato plants grown from vegetative cuttings or tubers may be developed under sterile conditions.

ORGANIZATION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

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