frequent phenomenon, I was very much interested to see that my theories were wrong, especially at the beginning of the causal series. Many of the corrugations that I had noticed were somewhat slanting, and now I saw that the scraping blade of the grading machine was responsible for the original vibration which was left in the road.

I have no doubt that the wheels of cars which travel on newly graded roads very much deepen these ridges when they resonate in tune to the original vibration of the scraping blade of steel which has left its marks in the ridges on the road. It must not be forgotten, too, that rain falling on the road will then also tend to drain off along these ridges and deepen them by erosion. I am wondering, too, when the road is of a certain elastic consistency, with a slight amount of moisture in the top layer, whether it will not then act much in the same fashion as the black asphalt pavements do when they are corrugated by impact, especially on down grades.

Naturally, this was only a single instance that came under my observation, but I made sure that there were no corrugations in front of the grader and that there were characteristically slanting and partially formed ones behind, and I am offering this bit of discussion in the hope that the matter may be verified or contradicted by observations of others. In general, this additional cause does not contradict the excellent explanation of Professor Dodd, but goes simply one step farther in certain cases.

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THE SEARCH FOR ELEMENTS ESSENTIAL IN ONLY SMALL AMOUNTS FOR PLANT GROWTH

FOR many years the essential nature of certain elements for normal plant growth remained undiscovered because they are needed in such small amounts that they were supplied as undetected impurities in the media in which the plants were grown. Between the years 1910 and 1919 Mazé,[1] by careful technique, showed boron, zinc and manganese to be essential to the growth of maize. Possibly because most of his papers were published in a journal devoted chiefly to bacteriological literature, they were overlooked by most plant physiologists. It was not until 1922 that the work of McHargue[2] emphasized the essential nature of manganese.

Boron was the next of these elements to receive attention. Warrington[3] in 1923, showed it to be essential for broad beans (*Vicia Faba*) and probably for runner beans, crimson clover (*Trifolium incarnatum*) and *Trifolium multiflorus*, but reported inconclusive results for white clover (*Trifolium repens*) and peas and negative results for barley and rye. The writer[4] in experiments with silicon and aluminum in which purified salts were used, confirmed the results with broad beans. The "mason" jars in which the solution culture experiments were being carried out were coated with "Valspar," a resistant varnish, to prevent contamination by solution of the glass, and sufficient boron for apparently normal growth of wheat, peas, millet and *Penisitum vilosum* was furnished by the varnish. Broad beans, however, made very little growth and showed the symptoms described by Warrington. They made remarkable recovery and normal growth when .5 mg. per liter of boron as boric acid was added to the solution. Later on when using uncoated jars in an experiment to determine whether or not chlorine is essential to plant growth, buckwheat failed to develop beyond the cotyledon stage when purified salts were used but developed normally when the ordinary "C.P." analyzed salts were employed. Because of the experience with broad beans, absence of boron was suspected of being the limiting factor. Investigation showed this to be the case. This and the effect of boron on the growth of sunflowers led the writer to study the effect of the absence of boron on a number of plants. Part of this work[5] with that continued at the University of California and later at the University of Minnesota, showed boron to be essential to corn, peas, sunflowers, vetch, barley, buckwheat, dahlias, lettuce, potatoes, millet, castor beans, sugar beets, kafir, sorghum, flax, mustard and pumpkins. Plants differed in the time, and to some extent in the way, in which the effect first appeared but none of the plants reached the flowering stage. Dicotyledonous plants in general responded more quickly than did monocotyledonous plants. In the case of sunflowers, cotton and buckwheat, the tops did not develop beyond the cotyledon stage and the roots grew very little. Other dicotyledonous plants showed the lack of boron by suppressed roots with enlarged apices within a few days but, depending on the type of plant, produced from

[1] Mazé, P. *Ann. Inst. Pasteur.* 1914, 28, 21–68; 1919, 33, 139–173. *Comp. Rend. Acad. Sci.* 1915, 160, 211–214.

[2] McHargue, J. S. *Jour. Amer. Chem. Soc.* 1922, 44, 1592–1598.

[3] Warrington, Katherine. *Ann. Bot.* 1923, 37, 629–672.

[4] Sommer, A. L. *Agri. Sci. Series,* Univ. California. 1926.

[5] Sommer, A. L. and Lipman, C. B. *Plant Phys.* 1926, 1, 231–249.

two to eight leaves. Soy beans were an exception; neither the tops nor the roots showed the effects of the lack of boron for two weeks. In this case also the roots were the first to show the effect. Monocotyledonous plants grew for a greater length of time and produced much better root systems than did the dicotyledonous plants. Many of these plants showed abnormal tillering as well as withering of the growing points of the tops. Corn showed the effects of the lack of boron in a week, but continued to produce small tillers for some time. Barley, under winter greenhouse conditions, apparently grew normally for a month, but after that the difference between the plants without boron and the controls developed rapidly. Bermuda grass (the only plant investigated which did not show marked injury in the absence of boron) is still under investigation, and so far has given very doubtful results. It is a very resistant grass and after an initial addition of iron to the culture solution, grew well and with no signs of chlorosis, without further additions of iron, during a period of two months while the writer was absent.

It is interesting to note that in a recent paper by Brenchley and Warrington,[6] buckwheat and potatoes are among the plants reported to have given inconclusive results and that peas completed normal development without boron. These results, as well as some reported in Warrington's earlier paper, are in marked contrast to those obtained by the writer. Whether this is due to a difference in technique or to the amount of boron stored in the seed is a point still to be investigated, but the fact that these authors obtained better growth without the addition of boron when they changed the solutions frequently suggests that the salts which they used may not have been entirely free from boron. In the case of the potato, the writer did not use seeds but allowed the tubers to sprout, removed the sprouts and transferred them to culture solutions.

Zinc, of the three elements mentioned above, is the one in which the conditions of experimentation must be most carefully controlled. The ordinary glass "mason" jar, in which many solution culture experiments are carried out, apparently furnishes all the zinc the plant needs. It was not until an attempt was made to use pyrex beakers with purified salts that the need of zinc was suspected. Solutions of the same salts which had produced good plants in ordinary glass failed when pyrex was employed. Wheat grew well for about two weeks and then stopped growing, turned yellow and finally died. The roots on the other hand were in good condition when the tops were dry and apparently dead. The analyses published by McHargue[7] in which zinc was found in seeds led the writer to try zinc which was found to be the limiting factor. It was not until the experiments with barley and sunflowers were completed that the paper by Mazé, in which he showed zinc to be essential for maize, was discovered in the literature. As in the case of the lack of boron, recovery from the lack of zinc can be accomplished by the addition of .5 mg. of the missing element per liter to the culture solution. Smaller quantities may be sufficient, but were not tried.

Zinc was shown to be necessary for barley, sunflowers, wheat, buckwheat, broad beans and red kidney beans. Buckwheat, sunflowers, barley and wheat showed the effects of the absence of zinc in the early stages of growth; wheat and barley died in the early stages, while some of the sunflowers and buckwheat plants, although much smaller than the controls, produced a few small flowers. The broad beans and red kidney beans without zinc appeared to grow as well as the controls until they reached the flowering stage. At this stage, the plants declined rapidly; most of the leaves fell off and only a few flowers on the broad beans developed. No seed was produced by the plants without zinc, while those with zinc developed normally.

When pyrex glass was used, silicon and aluminum, and later traces of other elements, including copper, were added to the solution. Barley failed to make good growth in pyrex containers unless silicon was added and there was some indication that copper is also necessary.

A study of these problems has shown that it is only by exercising the greatest precautions that we may solve the problem of essential elements. The salts must be repeatedly crystallized from pure distilled water (essentially conductivity water) and in some cases be derived from elements and acids or from other salts, for the "C.P." salts of trade usually contain, as impurities, sufficient amounts of certain elements to produce normal plant growth. Contamination by dust and in some cases other impurities in the air (for example, chlorine, in chlorine studies) must be carefully avoided. The type of container is also an essential factor. The ordinary glass jar must be avoided in many cases because of its solubility. Pyrex is suitable for most work, and, although a boro-silicate, does not yield sufficient silicon and boron for the normal growth of at least some plants. The effect of the lack of boron, how-

[6] Brenchley, W. E. and Warrington, Katherine. *Ann. Bot.* 1927, 41, 167–187.

[7] McHargue, J. S. *Jour. Amer. Soc. Agron.* 1925, 17, 368–372.

ever, appears more slowly than when ordinary glass is used. Containers other than glass will probably have to be employed before the whole problem of essential elements is solved.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CONTAINER FOR FIELD COLLECTION OF MOSQUITO LARVAE

IN the prosecution of malarial or mosquito studies larval collections play no small part. Containers used for captured larvae are subject to various disadvantages. For example, if the collecting jar is kept closed during field operations, the cover or cork must be removed whenever specimens are transferred to the container. If left open the contents are often lost because of jarring, especially if one is collecting in an area of irregular topography. Furthermore, most containers used for this purpose have either no mechanism for their attachment to the belt, or only an inadequate arrangement. The apparatus described below was devised to overcome the disadvantages just cited.

The container is a four-ounce jar with a mouth diameter of 40 mm. Two glass tubes with inner diameters of 4.5 mm. and 1.5 mm. run vertically through the rubber stopper as shown in the illustration. The outer termination of the former is flared

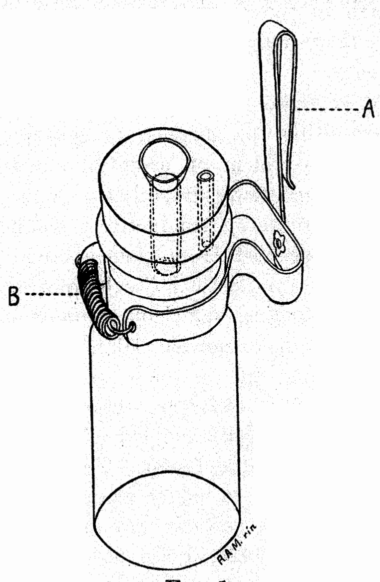

FIG. 1

into a funnel with a maximum diameter of 15 mm. and height not exceeding 10 mm. The inner end is flush with the surface of the stopper. The shorter the height of the protruding funnel the less will be the risk of breakage. The widened portion facilitates the transfer of larvae from the dipper in which they were captured, to the receptacle, by means of a pipette. The smaller tube practically prevents the formation of air bubbles in the larger. Its inner termination extends slightly beyond the stopper to prevent particles of the rubber cork from filling the tube and thus hindering air circulation.

The bent portion (A) made of nickel plated metal served to hold a key ring to a belt. It is now used for a similar purpose except that it is riveted to the collar, a piece of spring steel 13 mm. wide, so constructed that the jar is held tightly in place when its neck is enclosed within the collar. A hook similar to that shown in the illustration, except that it extended upward from the lower part of A, was cut off to better adapt the remainder for the design in view. The coiled spring (B), while not necessary, renders slipping of the jar impossible. All metallic parts should preferably consist of rust resisting material.

The apparatus after several months' trial in Porto Rico has proven fairly satisfactory. It is hoped that this descriptive note will stimulate others to improve the present model.

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DECALCIFICATION OF BONE IN ACID FREE SOLUTIONS

IN attempting to develop a method for the determination of an orthophosphate in bone, one of us observed that tertiary calcium phosphate is dissolved on addition of an excess of a magnesium citrate reagent even in the presence of a large excess of concentrated ammonia. White,[1] some four years ago, suggested the use of a solution of ammonium citrate for removing the lime salts from bone and the solvent action of the magnesium citrate reagent upon tertiary calcium phosphate suggested to us its possibilities as a decalcifying agent for histological purposes. The attempt to decalcify osseous tissue by means of this reagent proved successful.

The reagent is prepared as follows: Dissolve 80 gm of citric acid in 100 cc of hot water. Add 4 gm of magnesium oxide and stir until dissolved. Cool, and add 100 cc of ammonium hydroxide (density 0.90). Dilute to 300 cc, let stand 24 hours and filter. (If the magnesium oxide contains much carbonate, it

[1] White, C. P., *Jour. of Path. and Bact.*, Vol. 26, No. 3, 1923.