THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

VOL. 4. NOS. 1 AND 2.

MARCH AND JUNE, 1947

CARBOHYDRATE ABSORPTION BY ROOTS OF *PINUS TAEDA*.

By H. E. YOUNG, D.Sc.Agr., Pathologist, Science Branch, Division of Plant Industry.

SUMMARY.

A laboratory experiment demonstrated that mycorrhizal fungi are able to produce soluble carbohydrate from cellulose in sufficient quantities to support the growth of seedlings of Pinus taeda in sand culture in the absence of photosynthesis, and also that maltose in the absence of mycorrhizas can, as a soil nutrient, substitute for photosynthetic activity to some extent.

INTRODUCTION.

The part played by organic matter in the soil in the occurrence of fused needle disease of *Pinus* was dealt with in a previous paper by the author (Young, 1940a), and observations made in South Africa by Sherry (1941) emphasized the importance of organic matter in pine nutrition.

In order to obtain further information concerning the rôle of organic matter in pine nutrition, experiments were designed and carried out with the object of determining whether carbohydrates obtained from insoluble cellulose material can be made available to pine plants with the aid of their mycorrhizal fungi. Seedlings of *Pinus taeda* were used as test plants. An attempt was made to grow seedlings of this species under aseptic conditions in a carbon-dioxide-free atmosphere with various sources of carbohydrate supplied in the rooting medium. From this it was hoped that evidence concerning the carbohydrate relationships of pine roots would be obtained.

PRELIMINARY TESTS.

Prior to proceeding with the main experiment a reducing sugar was shown to be present in healthy mycorrhizas and absent from non-mycorrhizal roots.

A further preliminary test was carried out which involved the growth of *Boletus granulatus* and *B. viscidus* on pure cellulose. Both these species of fungi form mycorrhizas with *Pinus* (Young, 1940b). *B. granulatus* is one of the

most common mycorrhiza formers in Australia and is plentiful in Queensland. The cellulose (filter paper) was cut up and sterilized in Erlenmeyer flasks after the addition of a carbohydrate-free nutrient solution consisting of—

Potassium dihydrogen phosphate	0·1	gm.
Magnesium sulphate	0·169	gm.
Calcium chloride	0·054	gm.
Colloidal iron phosphate	0·1	ml.
Water	1·0	litre

Tests with Fehling's solution for the presence of reducing sugars in the medium were negative. The paper alone also gave a negative test.

After sterilization the flasks were inoculated with pure cultures of the two fungi concerned. The least possible amount of inoculum, which consisted of a small quantity of aerial hyphae in each case, was used. Ten flasks were inoculated with each species of fungus. The cultures were then incubated at 27° C. At the end of a fortnight samples from all flasks gave positive tests with Fehling's solution, and quantitative determinations of the reducing sugars in all cases in the remaining material were made in the chemical laboratory.

The experiment demonstrated that the two fungi—*B. granulatus* and *B. viscidus*—are able to digest cellulose and in the course of the process produce a reducing sugar. This supported the previously stated hypothesis (Young, 1940a) that the mycorrhizal fungi of *Pinus* are able to use cellulose which is supplied in forest litter and in the course of its decomposition produce soluble carbohydrates which may be used by the higher plant. The importance of the presence of fresh supplies of organic matter is shown by the fact that only in the unrotted condition is there an appreciable quantity of cellulose available.

It was also indicated that the growing of *Pinus* in a carbon-dioxide-free atmosphere might be possible.

MAIN EXPERIMENT.

In order to test the possibility of growing *Pinus* in a carbon-dioxide-free atmosphere an experimental design was devised whereby seedlings of *P. taeda* could be kept without carbon dioxide and their source of nutrients controlled. Three sets of conditions with respect to nutrient supply were imposed, viz.,

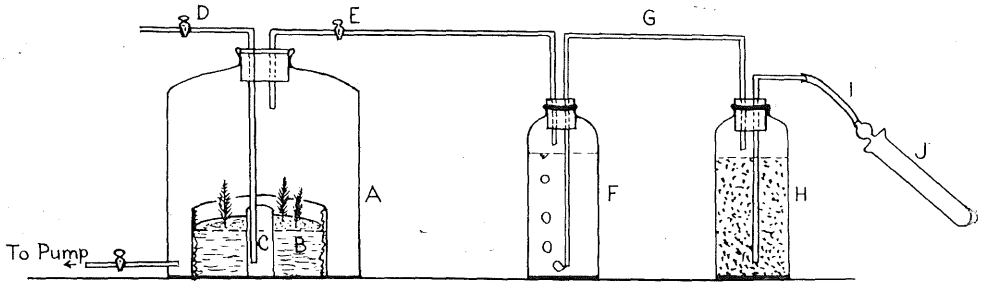
(1) Sand in glass dishes plus chopped filter paper (20 gm.) and carbohydrate-free nutrient solution as given on page 2.

(2) Sand, filter paper, carbohydrate-free nutrient solution and maltose (20 gm. per litre).

(3) Sand, filter paper, carbohydrate-free nutrient solution and inoculation with *Boletus granulatus*.

Filter paper as a source of available carbohydrate was common to all treatments.

The general design of the experiment will be seen on referring to the following diagram and description:—

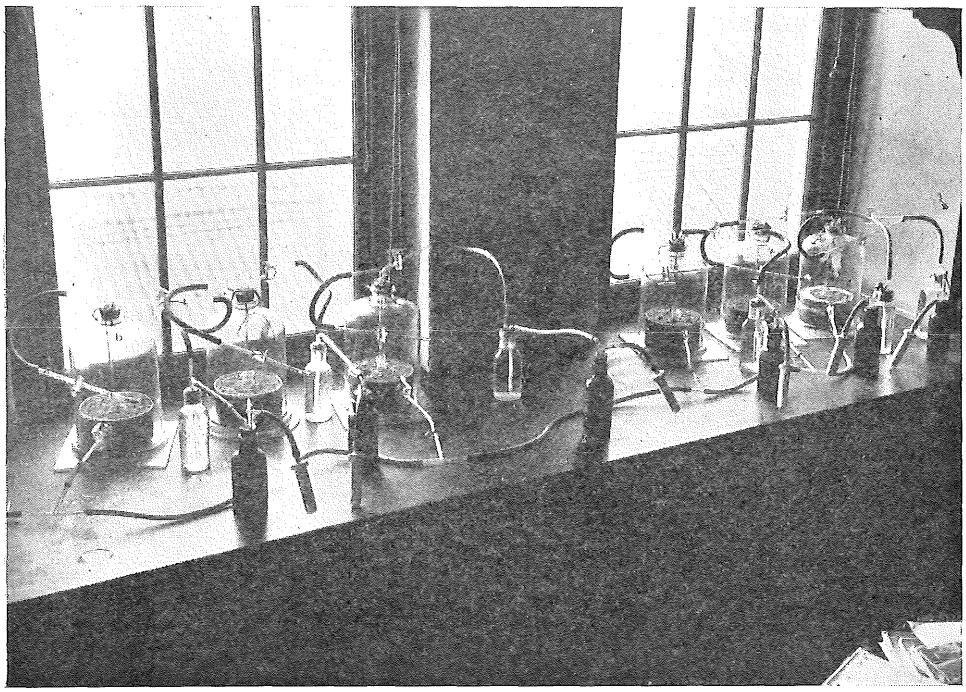


Diagrammatic representation of design of one unit of the carbohydrate experiment.

The bell jar A covered the glass dish B, which was 4½ inches deep and 7 inches in diameter and contained washed (acid and alkali then distilled water) river sand as a rooting medium. In the sand was placed a 1-inch diameter glass tube (C) with a gauze apron tied around the bottom. The tube had a serrated bottom in order to allow liquid to percolate in and out of the tube. The gauze was used to prevent sand from moving into the glass well C and interfering with the flow of fluids. A glass tube with a tap was fitted into the rubber stopper of the bell jar and arranged so that it came within half-an-inch of the bottom of the well C in the sand. A second glass tube with stopcock was arranged so as to pass a short distance through the stopper. This tube was connected by rubber pressure tubing to a bottle (F) by means of a glass tube just penetrating the stopper. This bottle contained a 5% solution of fresh sodium hydroxide plus 50 ml. of saturated solution of barium chloride, yielding baryta water. Another tube (G) was placed in the bottle so as to pass through the stopper to within half-an-inch of the bottom of the bottle and from this bottle by means of pressure tubing and a short glass tube through the stopper of the second bottle (H). This second bottle contained granulated soda lime. From H a glass tube (I) passed almost from the top up through the stopper and was connected by pressure tubing to a Berkfield-Chamberlain bacteria-proof porcelain filter (J). Through the side of the bell jar (A) as a means of exhausting the apparatus a glass tube with a stopcock was passed through a rubber stopper so as to reach just inside it. The other end of the tube was connected by pressure tubing to the filter pump. This exhaust tube was placed near the bottom of the apparatus in order to remove any carbon dioxide which might accumulate.

The object of the apparatus was to provide a means of aerating the bell jar with carbon-dioxide-free air. On operating the suction by means of the suction pump and opening all the stopcocks save that in the tube D, air was drawn through the filter and sterilized, then through the soda lime and freed of carbon dioxide, bubbled through the caustic soda-barium chloride solution and so into the bell jar and out into the pumping mechanism.

The baryta water solution acted as a check on the efficiency of the soda lime by producing a milky precipitate if and when any carbon dioxide got past the soda lime bottle. The rate of passage of air was governed by means of the pressure exerted by the suction pump and by the amount of opening to which the stopcocks were regulated. In practice it was run at three bubbles per second through the baryta water. Six such systems were set up as shown in the plate and all connected by glass T-pieces and rubber tubing to the one pump. The six sets could be worked simultaneously or in any combination and could be disconnected easily.



Battery of six CO_2-free jars with pine seedlings growing in nutrient solution.
[*Photograph by E. Suchting.*

All joints were sealed with Gurr's mounting medium. The bell jars were affixed to their ground glass bases by boiled Vaseline which was finally coated with euparol on the outside of the joints.

The apparatus was set up on a laboratory bench with no direct sunlight and arranged so that all bell jars received as nearly as practicable the same amount of illumination.

During the course of the experiment room temperatures varied from 70° to 79° F.

The complete apparatus was steam sterilized, six germinating seeds of *Pinus taeda* were placed in each glass dish under aseptic conditions, and the apparatus was closed and sealed.

Difficulty was experienced in obtaining sterile seed. This was overcome by removal of the testa and sterilization of the seed surface for 20 minutes in bleaching powder solution (10 gm. in 140 ml. water). The seeds were then germinated on potato dextrose agar in sterile Petri dishes. A large number of seeds was germinated in this way and when sufficient sterile seeds had radicles about 1 inch long they were planted out. Aseptic precautions were taken throughout. In treatment 3, involving the mycorrhizal fungus, the dishes at the time of seed planting were seeded with an inoculum of a fresh pure culture of the mycorrhizal organism, *Boletus granulatus*. (When *Boletus viscidus* was used as an inoculum it proved too vigorous and smothered the seedlings under the conditions of the experiment; *B. granulatus* was eminently satisfactory.)

For the first 10 days ordinary sterilized air was pumped through the apparatus continuously but without passing through the soda lime and baryta water. At this time the cotyledons of the seedlings were visible. After this initial period carbon-dioxide-free air, obtained by placing the soda lime and baryta water bottles in the circuit, was used. By continuous pumping, the carbon dioxide in the bell jars at the end of the initial period and subsequently any carbon dioxide respired by the mycorrhizal fungi on the plants was removed. Periodic checks for carbon dioxide in the exhausted air were made with the aid of baryta water.

Difficulties due to contamination of the dishes by foreign organisms were experienced but finally overcome. These contaminations occurred at the planting of the seed and were usually associated with the seed itself.

At 21-day intervals the dishes were freshly irrigated with their respective sterile nutrient solutions and then drained again. This was effected by passing sterile nutrient solution into the dishes by means of the irrigating tube D. The flow was induced by manipulating the stopcocks and drawing the fluid in by means of the suction pump. The dishes were flooded and the excess solution then removed by means of the glass wells (G), which allowed any free solution to be siphoned from the sand in the dishes back into the flasks of nutrient solution.

Observations.

The appearance of the seedlings as brought about by each of the treatments was as follows:—

1. *Soluble carbohydrate-free, mycorrhiza-free nutrient.* Seedlings were spindly, grey-yellow-green in colour; the cotyledons were reflexed and the plants gradually wilted. No primary needles developed. The seedlings soon lost the power of supporting their own weight and finally shrivelled and died. Death commenced at the cotyledon tips. Mean life was 59 days.

2. *Maltose added—no mycorrhizal fungus.* Good growth but not so vigorous as 3. Plants became marked by punctate depressions on the cotyledons and primary leaves, which were somewhat distorted. This was possibly due to too high a concentration of the nutrient solution. Growth gradually ceased. Primary leaves appeared at 29 days. Mean life was 102 days, with one plant still living at 140 days, but with poor vigour. The roots of the dying plants

persisted in the living state for a considerable period after the death of the tops. The behaviour of these sugar-fed plants accords with the results obtained by Knudson and Lindstrom (1919), who attributed the poor growth of albino corn plants in a nutrient containing carbohydrate to the slow diffusion of the sugar. For this reason the plants were unable to absorb a sufficient quantity of organic material to supply the needs of the entire plant, the roots alone being sustained. The presence of mycorrhizas is not mentioned by the authors and it is, therefore, assumed that they were absent.

3. *Soluble carbohydrate-free; mycorrhizas present.* Good growth; some deaths from smothering by overgrowth of the mycorrhizal fungus. Primary leaves were produced in 21 days, that is eight days before those plants treated with carbohydrate in the absence of fungi. Mean life was 270+ days. Six plants were still growing vigorously with no signs of decline at the time of completion of the experiment. The remainder were smothered by fungus growth in their early stages. Mycorrhizas were present on the roots of the seedlings.

The experiment was repeated and the observations on the second series agreed with those on the first series as described. As each treatment was duplicated in each series, four replications of each treatment were carried out with similar results. In the second series the aeration treatment was modified to some extent. It was discontinuous and carried out only for one hour per day for the first 42 days and then continuously again. This was done because of inability to obtain adequate supplies of soda lime owing to wartime difficulties. This was regrettable, since fungal respiration of carbon dioxide in the mycorrhiza-inoculated jars would not be continuously eliminated. However, the two series corresponded in their reactions and with the resumption of continuous aeration no change was noted, showing that this factor was not serious.

CONCLUSIONS.

The experiment showed that seedlings of *Pinus taeda* can exist and grow in a carbon-dioxide-free atmosphere and that the fungus *Boletus granulatus* is capable of providing pine roots with a carbohydrate supply derived from cellulose and suitable for pine nutrition. Maltose supplied in solution also supports growth much longer than sugar-free media but not for so long and not so well as that provided by mycotrophic means.

It was also shown that mycorrhizas with the aid of organic matter are able to supply carbohydrate to the plants by means of the roots and that this supply can be at least accessory to that supplied by photosynthetic activities.

REFERENCES.

KNUDSON, L., and LINDSTROM, E. W. 1919. Influence of sugars on growth of albino plants. Amer. J. Bot. 6: 401-406.

SHERRY, S. P. 1941. J. So. Afr. For. Assoc., 6, 102.

YOUNG, H. E. 1940a. Fused needle disease and its relation to the nutrition of *Pinus*. Qld. Agric. J. 45 (1): 45-54; (2): 156-177; (3): 278-315; (4): 374-392; (5): 434-453.

YOUNG, H. E. 1940b. The influence of different species of mycorrhiza-forming fungi on *Pinus* and *Araucaria*. J. Aust. Inst. Agric. Sci. 6 (1): 21-25.