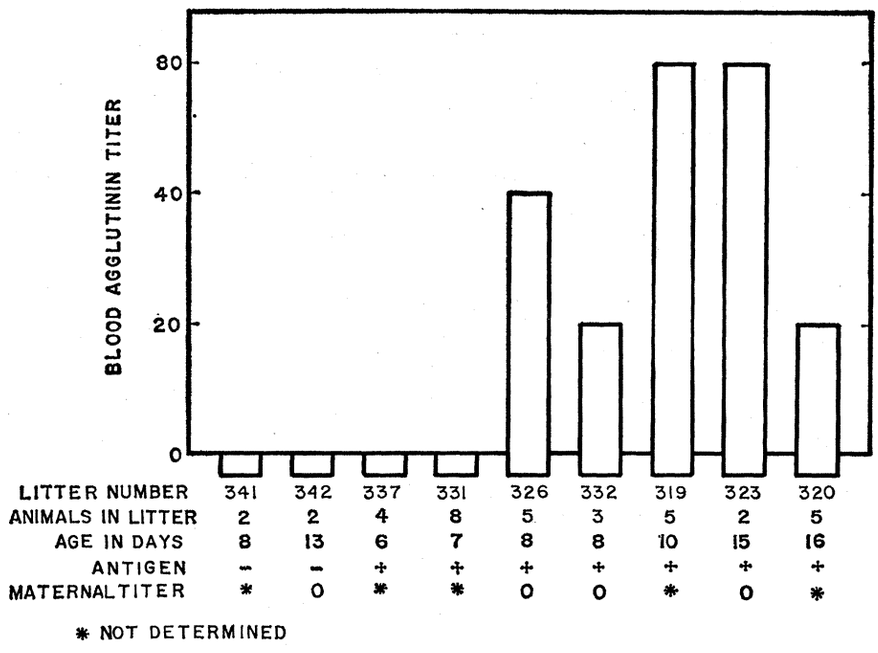


Fig. 1. Agglutinin titers of immunized and nonimmunized opossum embryos and of randomly selected mothers. Each bar represents the titer of a pool of heparinized blood from the number of embryos indicated. Titers of maternal serum are recorded in the bottom line of the figure. Each titer is expressed as the reciprocal of the highest dilution of serum in saline which gave a weak agglutination as judged by visual examination after resuspension. Serums giving no agglutination at the lowest dilution were considered negative.

injection, and heparinized blood from one litter was pooled to obtain a volume sufficient for agglutinin titration. Antibody titration was carried out by the method of Cannon *et al.* (*9*).

An antibody response was obtained when the antigen was injected on the 8th day or thereafter. No measurable titer was present in animals injected before the 8th day. To exclude the possibility that the antibody measured was of maternal origin, serums from mothers selected at random from both immunized and nonimmunized litters were taken for titration. Serums from noninjected opossum embryos were also titrated. All these controls gave negative results (Fig. 1).

These observations suggest that thymic or lymph node lymphoid tissue, or both, are required for an immune response to a flagellar antigen of *Salmonella typhi*. This phenomenon is particularly important in view of the role of the thymus in immunogenesis recently proposed (*10*). In the time covered by this experiment, namely between the 6th and 16th day, splenic lymphoid tissue, plasma cells, and secondary lymphoid nodules have not yet appeared in normal opossums (*7*).

Our work then extends previous work (*5*, *6*) to an earlier stage in embryonic development and indicates that antibody can be formed by embryos immediately after the appearance of lymphoid tissue in the thymus and in the lymph nodes. The observation that embryos can respond to an antigenic stimulus by forming antibody is thus emphasized and suggests these questions: (i) is this response accompanied by a proliferation of cells which normally develop only in more mature animals, (ii) if so, what cell types are involved (*11*)?

MARIANO F. LA VIA
DAVID T. ROWLANDS, JR.
MATTHEW BLOCK
*Departments of Pathology* and *Medicine, University of Colorado Medical Center, Denver 20*

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1 April 1963

## Endotrophic Mycorrhizae Influence Yellow Poplar Seedling Growth

Abstract. *Yellow poplar seedlings infected with endotrophic mycorrhizal fungi grow much faster than seedlings grown without mycorrhizae. A method of pot culture that uses natural soil structure provides an excellent means of studying growth differences due to microorganisms.*

Studies begun in 1959, designed to show effects of various soil factors on tree seedling growth, clearly indicate that endotrophic mycorrhizal fungi are important for the vigorous growth of seedlings of the yellow poplar, or tulip tree (*Liriodendron tulipifera* L.). Uninfected plants were small and chlorotic, but plants infected with the mycorrhizal fungi were large and vigorous.

The well-known ectotrophic mycorrhizae are comprised of a fungal sheath of plant roots and hyphal penetration between cortical cells. Endotrophic mycorrhizae do not form a fungal sheath and hyphae grow inside the cortical cells. Past research has shown that ectotrophic mycorrhizae are important in plant nutrition. However, work on the functions of the more common endotrophic mycorrhizae has been very limited. Mosse (*1*) in England found that inoculation of apple cuttings with an *Endogone* species from strawberry roots produced endotrophic mycorrhizal infection and generally greater growth. Baylis (*2*) used the soil under *Griselinia littoralis* (Cornaceae) to inoculate seedlings grown in sterile soil to show that trees infected with fungus producing vesicular-arbuscular mycorrhiza were bigger after 1 to 2 years' growth.

In the yellow poplar studies samples of undisturbed soil were taken in 1-gal tin cans driven into forest soil (*3*). The large plugs or cores in the tin containers served as excellent media for growing

seedlings with soil structure essentially intact. The sample site is in a mixed hardwood stand in southern Indiana. The soil is classed as Wellston silt loam and the *p*H is about 6.

Autoclaving and gassing with methyl bromide were used for sterilization, and both methods proved satisfactory. For convenience, gassing was used in most of the tests. Both small plugs of natural forest soil and macerated roots of forest-grown yellow poplar seedlings were used to supply inoculum for sterilized containers. The yellow poplar root inoculum was prepared by washing roots thoroughly in distilled water, washing them in a 0.5-percent water solution of sodium hypochlorite, and re-washing in distilled water. Then the roots were cut and macerated in a mortar. This procedure was followed to decontaminate the root surfaces as much as possible. About 2 to 3 g fresh weight of macerated roots were added to each container. Sections of roots used for inoculum were examined and found to be infected with endotrophic mycorrhizal fungi.

The macerated roots used in this study are not a pure inoculum. There is the possibility that other microorganisms remain as contaminants on the root surfaces even after washing in sodium hypochlorite. Unfortunately, techniques for producing pure inoculum of endotrophic fungi are not yet available. Other known work with the endotrophs has had the same general deficiency as the present study. For example, in a German study with corn (*4*) the inoculum used was corn roots.

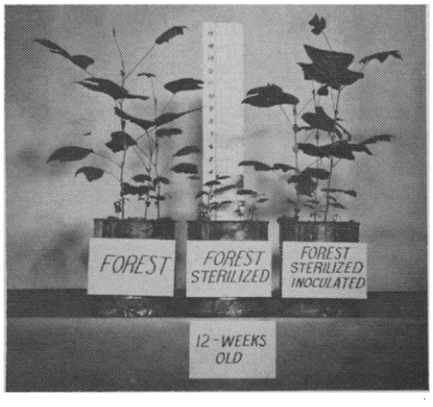


Fig. 1. Seedlings of yellow poplar, tulip tree (*Liriodendron tulipifera* L.) grown for 12 weeks in undisturbed soil cores from a forest site demonstrated that endotrophic mycorrhizal fungi influence growth. (Left) Seedlings grown in unsterilized soil were infected. (Center) Seedlings grown in sterilized soil were nonmycorrhizal and nonvigorous. (Right) Seedlings in sterilized soil inoculated with yellow poplar roots were mycorrhizal.

Yellow poplar seeds were germinated in trays and planted in the containers of undisturbed soil. The seedlings were grown on a 14-hour day under artificial light for 12 weeks. Distilled water was used for watering. Three replications (containers) of each treatment were ample to demonstrate statistical differences in seedling size in each of four separate studies. Seedlings were recovered nearly intact from the containers by soaking and washing. Examination of the root systems for mycorrhizal fungi was made by Hacskaylo (*5*).

Growth differences among the various treatments were outstanding (Fig. 1). In the most recent study total fresh weight of roots and tops averaged 1.6 g per seedling in sterilized containers. In contrast, seedlings from unsterilized containers and sterilized containers inoculated with macerated yellow-poplar roots averaged 7.7 and 9.0 g, respectively. Microscopic sections of the root systems revealed that the seedlings from sterilized containers were nonmycorrhizal, while seedlings from unsterilized and sterilized-inoculated containers were mycorrhizal.

It is interesting that the influence of the fungus was not effective immediately. At 7 weeks there was no height difference between seedlings in sterilized and sterilized-inoculated containers. Evidently the organism or the host plant must reach a certain stage of development before the mycorrhizal infection becomes effective.

Soil structure greatly influenced seedling growth. Moist soil from the sample site was sieved through a ⅜-inch-mesh screen and potted. Coarse litter and roots were discarded from the sieved soil, otherwise the sieved and undisturbed soil samples were identical except for porosity. After 12 weeks, seedlings in the sieved soil weighed only a sixth as much as seedlings grown in undisturbed forest soil. Seedlings in sieved soil were only 3.1 inches tall compared with 10.3 inches for seedlings in undisturbed soil. Sieving did not exclude mycorrhizal development but the infection was slight. So the successful demonstration of the influence of mycorrhizae on yellow-poplar growth was due primarily to the use of undisturbed natural soil.

The influence of endotrophic mycorrhizae on the growth of yellow-poplar was clearly demonstrated. Nutritional studies of other plants normally infected by endotrophs should be designed to evaluate the relations between the host plant and associated fungi. The use of containers with the soil structure essentially undisturbed provides optimum growing conditions and an opportunity for maximum differences among experimental treatments.

F. BRYAN CLARK
*Central States Forest Experiment Station, Forest Service, U.S. Depdrtment of Agriculture, Bedford, Indiana*

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## Tracks of Charged Particles in High Polymers

Abstract. *Heavily ionizing particles create trails of damage as they move through materials. In both addition and condensation polymers these trails can be selectively dissolved so that the sites and the directions taken by the moving particles are revealed. These materials thus serve as simple detectors of heavily charged particles.*

In many crystalline solids irradiation by heavily ionizing particles such as fission fragments produces trails of damage (*1*). This may be revealed by preferential chemical attack, that identifies the sites of damage by etch pits (*2*). Long, narrow etch channels may be produced (*3*) which identify the direction of the damage trail as well as the location of the damage. These phenomena occur also in inorganic glasses (*4*) and hence are not restricted to crystalline solids. Here we describe the observation of charged particle tracks in several organic high polymers.

We have demonstrated the particle-etching effect by bombardment with several high-energy, massive particles—including 400 Mev argon ions (*5*). The most convenient and rapid procedure, however, is irradiation of a selected area with fragments from the spon-