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Culture of Excised Embryos of Pinus Ponderosa

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Discussion

The thermoelectric method enabled the detection of upward movement of xylary fluid in elm branches under conditions favoring normal rates of transpiration. In our opinion, possible downward movement of branch fluid also was indicated in a limited number of trials. Further refinement of the technique is needed in order to determine definitely the location and magnitude of this downward movement. Relationships between rapid colonization of elm trees by *C. ulmi* and possible downward movement of xylary fluid remains unknown.

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Culture of Excised Embryos of Pinus Ponderosa[1]

Virgil K. Howe[2]

Abstract. Embryos of *Pinus ponderosa* Laws. were excised and placed on a nutrient agar medium to which auxins in varying concentrations had been added. Results indicated that when the auxins were dissolved in and diluted with 40 per cent alcohol there was inhibition of seedling growth and development. Techniques were devised which eliminated the use of alcohol. In the absence of alcohol, morphologically normal seedlings were produced which were transferred to quartz sand after 60 days and at 113 days were transplanted into soil. Apparent normal growth and development continued throughout the duration of the experiment. The addition of auxins produced no significant stimulation to the growth and development of the seedlings.

[1] From a thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biology from the University of New Mexico, Albuquerque, New Mexico, 1961. Sincere appreciation is due Dr. Loren D. Potter and Dr. Eugene W. Rypka for their advice and encouragement during the course of this study.

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The culture of plant embryos has become a useful tool in a number of biological studies. Embryo culture was employed in this study to observe the effects of various concentrations of five auxins upon the growth and development of seedlings from excised embryos of ponderosa pine (*Pinus ponderosa* Laws.).

The first successful plant embryo cultures were made nearly 60 years ago (Hannig, 1904). Rappaport (1954) has written a comprehensive review of the literature concerning plant embryo cultures. A great portion of the investigations has been carried out using embryos of angiosperms. Embryos of only a few gymnosperm species have been utilized; and when used, reports of the results are often sketchy.

One of the more thorough accounts of an attempt to culture embryos of a gymnosperm species was reported by Sacher (1956). Working with post germinal embryos of the sugar pine (*Pinus lambertiana* Dougl.), seedlings were produced that were inferior in growth to normally germinated seedlings and which would not grow in culture beyond two or three weeks. A few other investigators (Loo and Wang, 1943; Sterling, 1949; Haddock, 1942; Stone, 1948) have reported attempts of embryo cultures for several coniferous species with varying degrees of success.

Embryo cultures are divided into two caregories; pregerminal that utilize embryos of only a few cells and postgerminal that involve the use of mature or nearly mature embryos. My study used excised, postgerminal embryos of the ponderosa pine. The development of seedlings on an agar medium, the effects of their transfer to quartz sand and eventually to soil were studied.

Materials and Methods

The basic nutrient medium for the experiments consisted of an inorganic salt solution, a carbohydrate, and agar. The inorganic salt solution was prepared according to White (1954). Sucrose was chosen as the carbohydrate, and Difco "Noble" agar was included to provide a semi-solid medium.

The original scope of this study included the addition of various auxins at varying concentrations into the basic medium. Naphthalene acetic acid, indolebutyric acid, 2, 4-dichlorophenoxyacetic acid, gibberellic acid, and indoleproprionic acid were used. Concentrations of auxins were 100, 10, 1, 0.1, and 0.01 ppm. The auxins were dissolved in and diluted with 40 per cent (v/v) ethyl alcohol and incorporated into the basic medium. The medium was distributed in 5.0 ml portions to shell vials (21 x 77 mm), autoclaved, and slanted. Two sets of checks were prepared; an auxin check consisting of the basic medium plus

the amount of 40 per cent alcohol used as auxin solvent, and an alcohol check which differed only in the addition of water in place of alcohol.

The excision of the embryos and their transfer to the culture vials was done under strict asceptic conditions. The cultures were kept in the greenhouse throughout the duration of the experiment. Conditions were maintained as near optimum as possible.

Measurements were made using a graduated ocular disk that was calibrated in a binocular dissecting microscope. The measurements were taken at 5, 10, 15, 25, 40, and 60 days.

It soon became evident that the alcohol was detrimental to the growth of the seedlings. The three best auxin groups were repeated using the same procedures as previously described except that the auxins were dissolved in water. Dissolution was accomplished by placing the water-auxin mixture in the autoclave and allowing the pressure to rise to 22 lb./in.^{2} at a temperature of 121-123°C. The autoclave chamber was immediately decompressed; and before cooling, the auxins were diluted to the desired concentrations. These dilutions failed to show visible recrystallization and were deemed acceptable for use.

Test tubes (25 x 150 mm) were used instead of shell vials in this experiment in order to provide additional nutrient medium and space. However, the excellent growth of the second set of cultures created a space problem and necessitated the transplantation of the seedlings to washed quartz sand. A complete nutrient solution (Kurtz, 1956) was used while the seedlings were in sand. Eventually the seedlings were transferred to soil.

Results and Discussion

The cultures were observed periodically, and a comparison of the two check groups at the 15-day observation period revealed the inhibitory effect of alcohol upon seedling growth and development (Figure 1). When an auxin was also present in the medium, there was even greater inhibition (Figure 1).

Following the 15-day observation period, the decision was made to repeat the best three auxin groups using techniques which eliminated the use of alcohol. These experiments produced seedlings that appeared to be morphologically normal. Ninety-five per cent of the seedlings were maintained in tube cultures on agar medium for 60 days. They were transplanted in washed quartz sand to which a nutrient solution was added periodically. Measurements of the seedlings while they were maintained in sand indicated continued growth. After 53 days in the sand, the seedlings were transplanted to soil where good growth and development continued.

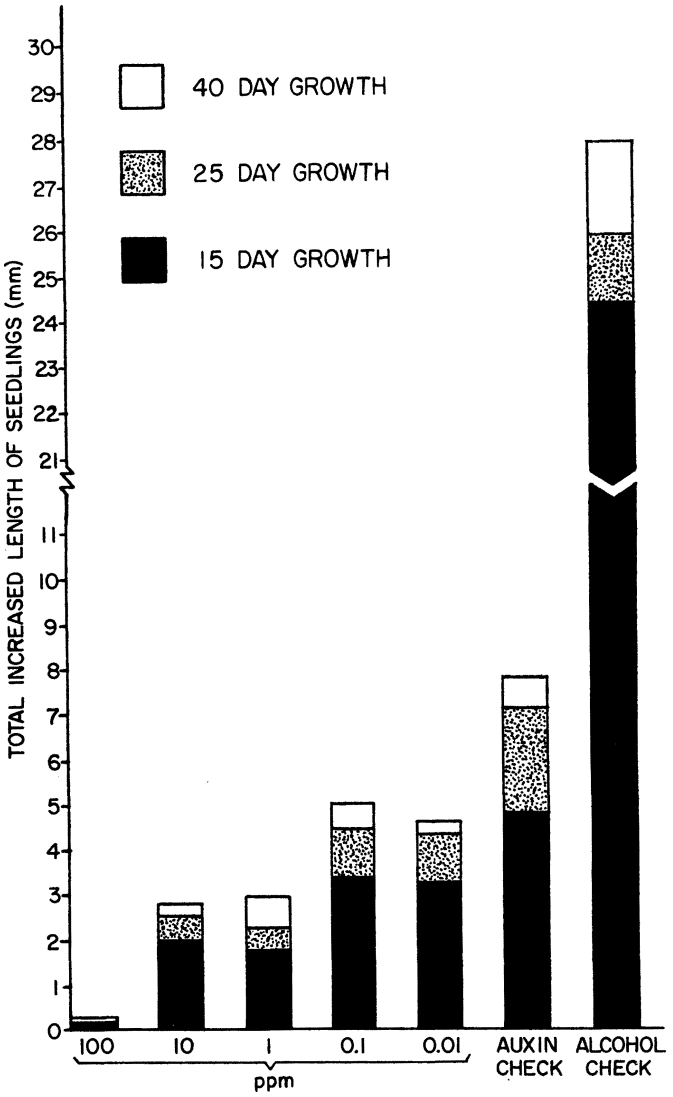

Figure 1. Comparison of one auxin series and the two checks (Total increased length of the seedlings at the time of observation expressed) (Courtesy R. Albertson).

Measurements of the experimental and check groups were compared. Two of the experimental groups appeared slightly stimulated (Figure 2); but the differences were not statistically significant using the t-test.

The results of the latter groups indicated that normal ponderosa pine seedlings can be produced with great frequency on an agar medium from excised embryos. Such success is achieved

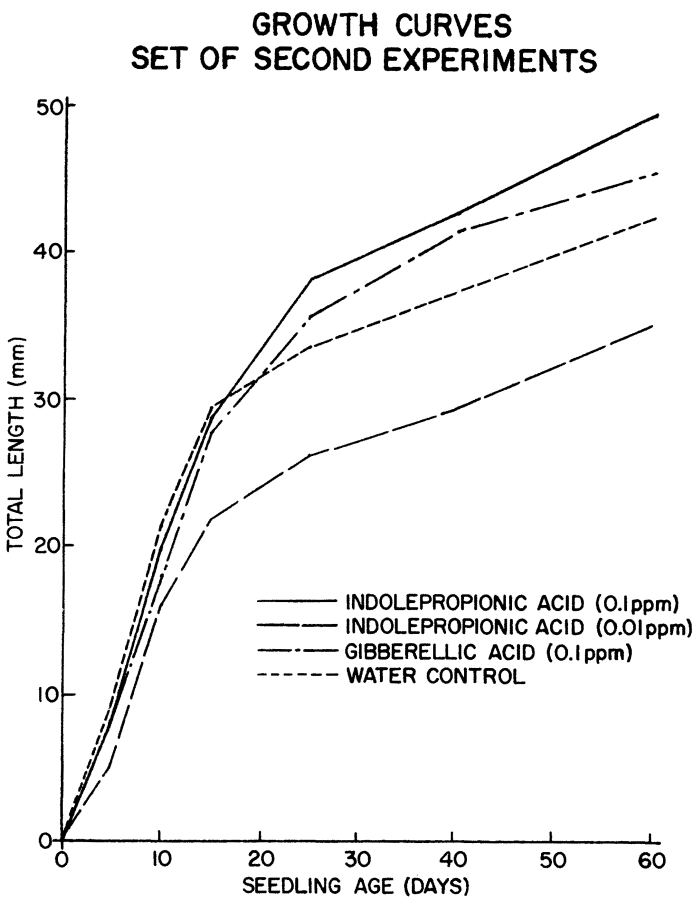

Figure 2. Growth of seedlings when alcohol was absent from the medium (Courtesy R. Albertson).

only when alcohol is eliminated from the medium. While no significant differences were noted with respect to the presence of auxin in the medium, a repeat of the first experiments using the modifications of the second and including greater numbers of replicates might prove worthwhile.

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Development of Axillary Buds of the Tillers of Zea[1]

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Abstract. An inbred line of popcorn and a line that contained teosinte germ plasm were compared with respect to the inflorescences of the numerous tillers produced by plants of these lines. In both lines, all terminal and axillary apices of the tillers initiated inflorescences. No axillary tassels were found on the tillers of either line. On a popcorn tiller, a terminal inflorescence was either a tassel, a tassel that bore a few scattered kernels, an ear, or an ear that had basal tassel branches. Every tiller of the "teosinte-contaminated" corn had a terminal tassel.

The recent revival of interest in the development of axillary buds of *Zea* has been due in part to agronomic considerations, in particular the production of commercially valuable hybrids that produce more than one harvestable ear on a plant. The breeding program has thus called for a search for multiple-eared types of maize as possible sources of the desired germ plasm. Some lines of popcorn are typically multiple-eared and also exhibit considerable tillering, a character that is undesirable in field corn. The introduction of germ plasm of teosinte, *Euchlaena mexicana* into *Zea* has yielded plants that have many ears and many tillers. These characteristics suggested examination of the shoot apices, especially the apices of the axillary buds, as well as all meristematic apices of tillers.

The structure of the pistillate inflorescence of maize had been described in detail in past years, and the extensive literature on the subject has been reviewed by Bonnett (1948), and Kiesselbach (1949). Studies that were related to specific agronomic

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