through 13 subcultures (Fig. 4). Growth has been most vigorous on a modified White's medium (*2*), to which 0.25% yeast extract and 1 mg IAA have been added per liter. Tissue proliferation continues either on agar or in shake culture at a rate which permits subculturing 1 : 8 in 3 weeks. Moreover, since its establishment as a tissue culture, the pollen tissue has shown no diminution of growth activity.

FIG. 4. *Ginkgo* pollen-derived tissue: this is one month's growth in an 125-ml Erlenmeyer flask; the original inoculum was 5 mm cube. (Natural size.)

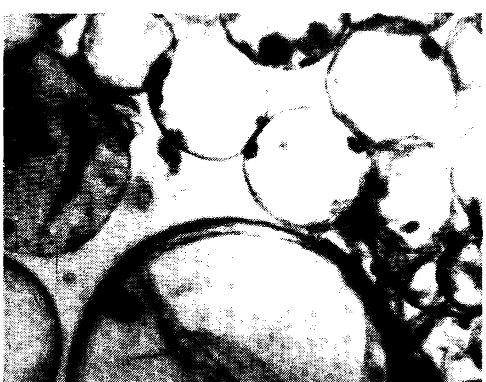

FIG. 5. Vacuolate tissue cells: the giant cell is 483 μ in diameter with clusters of 7 and 3 nuclei.

Ginkgo pollen tissue may be characterized as an undifferentiated, parenchymatous, and often multinucleate cell mass (Fig. 5). The tissue originally has a haploid complement of 12 chromosomes but later becomes polyploid.

Up to the present time, over 25 tissue initials have been observed in a total of 634 culture bottles; this is an incidence of tissue formation of about 4%. Subculturing of many of these initials has resulted in tissue proliferation, although only three such subcultures have been carried on as continuous clones. *Ginkgo* pollen tissue has thus demonstrated, repeatedly, a capacity for potentially unlimited explanation.

Growth *in vitro* of the female gametophyte of *Ginkgo* has also been obtained. In this case, marginal meristems are formed and they produce usually nodular outgrowths. And, although the initial inoculum of the female gametophyte tissue may increase sixfold in volume through marginal proliferation, excision and subculture of the outgrowths have, thus far, not been successful. When grown in light, the tissue not only retains its chlorophyllous nature, but also exhibits a marked increase in the intensity of pigmentation.

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A Report on the Waxy Constituents of Spanish Moss, *Tillandsia usneoides* L.

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Tillandsia usneoides L., commonly called Spanish moss, long moss, crape moss, and Florida moss, is a true epiphyte which festoons trees of swamps and hammocks of the southern coastal states.

The hard, resilient inner fibers are used extensively in the upholstery industry, the remainder of the plant being discarded or utilized as compost. Webber *et al.* (*1*) reported the presence of an antibacterial substance. Mayo Clinic studied the use of moss as a surgical dressing as it is more absorbent than cotton (*2*).

In view of the recent endeavors to procure a suitable substitute for carnauba wax from natural plant sources or by synthesis of low molecular weight waxes (*3*), attention is called to the wax present in commercial quantities in Spanish moss.

In his study on the carbohydrate constituents of Spanish moss, Schroger (*4*) reported the presence of a green-colored wax melting at 79 to 80° C. The presence of this wax was confirmed, and a constituent exhibiting steroidal characteristics was extracted and shown to possess estrogenic activity (*5*). The freshly gathered moss contains approximately 5% wax. The iodine number of this wax is 33.0, the saponification number 120.4, the acid number 25.0, the ester number 95.0, and the melting point 79–80° C.

This wax is soluble in various organic solvents, easily purified, and imparts a hard glossy finish to woodwork and leather, comparable to commercial waxes.

In view of the abundance of Spanish moss, the proximity of the supply and the economy of utilizing the waste material from the processing of upholstering fibers, it may prove profitable for some industrial organization to investigate this plant as a possible source for a hard natural wax.

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Effects on Plant Growth of Some Compounds with Structural Similarities to Maleic Hydrazide[1]

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Investigation of the effects on plant growth of compounds structurally related to maleic hydrazide should help clarify the mechanism of maleic hydrazide inhibition of plant growth. Maleic acid, maleimide, and the cyclic hydrazides and imides of related dicarboxylic acids should be particularly useful, although other hydrazides, hydrazines, and hydrazine itself might yield information of value. The difficulty of obtaining many of these compounds has been at least partially responsible for lack of reports on their effects on plant growth. This paper deals with the first of a series of experiments on the effects of such compounds on plant growth. Additional compounds in the groups mentioned are being investigated as they become available, not only for comparison with maleic hydrazide, but also for possible interesting effects which they may have on plant growth.

The effects of hydrazine derivatives, other than maleic hydrazide, on plant growth have been investigated little if at all, but there have been a number of studies on the effects of maleic acid and maleates on both plants and animals. Most investigators seem to agree that maleic acid is a respiratory inhibitor in animals and microorganisms, though some have reported it to be utilized as a respiratory substrate (*1, 2*). Annau (*3*) claims that maleic acid competes with fumaric and succinic acids for enzyme surfaces without fulfilling the functions of the latter acids and Weil (*4*) reported that maleic acid inhibits the Krebs cycle but not glycolysis. Copisarow (*5, 6*) found that maleic acid dissolved in etherial oils (but not in water) inhibited the sprouting of potatoes, the ripening of apples, and the growth of fungi on both. Isaacs (*7*) confirmed its effect on fruit ripening, but reported that skin injuries by the acid made the fruits more susceptible to fungi. English *et al.* (*8*) stated that maleic acid is slightly effective as a traumatin. Bonner and Galston (*9*) found that maleic acid had a slight inhibiting effect on plant growth, while Lundegårdh (*10*) reported that maleic as well as fumaric acid was utilized by wheat roots and accelerated their respiration. Thimann and Bonner (*11*) found that maleic as well as other organic acids counteracted iodoacetate growth inhibition. Krishnamurti and Subrahmanyan (*12*) found that maleic acid and a variety of other compounds inactivated the milk-clotting and protease enzymes of fig latex by affecting active —SH groups in the enzymes. Since Greulach and Atchison (*13*) and a number of subsequent investigators have found maleic hydrazide to be an antimitotic in plants, it is particularly interesting that Friedman *et al.* (*14, 15*) found maleic acid to be an antimitotic in chick fibroblasts and later (*16*) reported maleimide and citraconimide to be even more effective antimitotics, whereas succinimide was inactive. They suggest that antimitotic effectiveness was directly proportional to the rate of —SH uptake by these compounds.

Although hydrazides other than maleic have not been used on higher plants, Grunberg and Schnitzer (*17*) and others have found isonicotinic hydrazide and its derivatives to inhibit growth of the tuberculosis bacillus and claim it to be effective in the treatment of tuberculosis.

In the experiments reported on here bean and sunflower plants two weeks old and tomato plants three weeks old were dipped in solutions of the following compounds: maleic hydrazide, maleic acid, diformyl hydrazine, phenylhydrazine hydrochloride, succinic hydrazide, succinimide, isonicotinic hydrazide (rimifon), and 1-isonicotinyl 2-isopropyl hydrazide (marsilid).[2] Succinic and fumaric acids were also used for comparison with possible maleic acid effects, although all three acids were actually applied as their sodium salts to avoid pH effects. With the following exceptions, all solutions were 0.015 M: (1) in one experiment with beans 0.03 M diformyl hydrazine was used, (2) the quite insoluble succinic hydrazide was applied as a saturated solution of around 3×10^{-4} M, (3) succinic hydrazide was also applied to one series of plants at 4000 ppm in lanolin applied to the under surface of one leaf of each plant, with plain lanolin being used on the controls. Dreft was added to each solution at a concentration of 0.025% as a wetting agent. A minimum of six plants was used per treatment, the plants being maintained in a greenhouse in porous clay pots. Weekly observations and measurements of height were made, and "t" values were calculated to determine the significance of the differences of the means.

The effects of the various compounds on the growth

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[2] The maleic hydrazide (as the diethanolamine salt) was supplied through the courtesy of the Naugatuck Chemical Company, the rimifon and marsilid by the Hoffmann-LaRoche Corporation, and the succinic hydrazide, succinimide, and diformyl hydrazine were synthesized by the Chemistry Department of the University of North Carolina under the direction of Arthur Roe.