SUBMERSION INCREASES ETHYLENE AND STIMULATES ROOTING IN CUTTINGS[1]

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In 1933, Zimmerman and Hitchcock (5) of the Boyce Thompson Insititute reported that unsaturated hydrocarbon gases, including ethylene, stimulated initiation of roots in 15 species of herbaceous and woody plants. In willow, the gases also stimulated the growth of latent root primordia. Cuttings were exposed to different concentrations of the gases ranging from 10^{-4} to 1% under airtight containers.

According to our results, exposure of willow cuttings to ethylene gas stimulated root formation within an exposure time ranging from 0 to 30 min. Treatment for longer than 30 min. was less effective (Fig. 1).

Ethephon, recently developed by Amchem Co., has been used in many fields of agriculture. After being absorbed by the plants, the compound undergoes decomposition and releases ethylene gas to the plant tissues. Figure 2 indicates the root-forming effect of Ethephon on willow cuttings. The most effective concentration was 880 ppm. The effect of Ethephon on root formation of tomato plant is shown in Figure 3. Roots appeared all the way along the stem of the cutting.

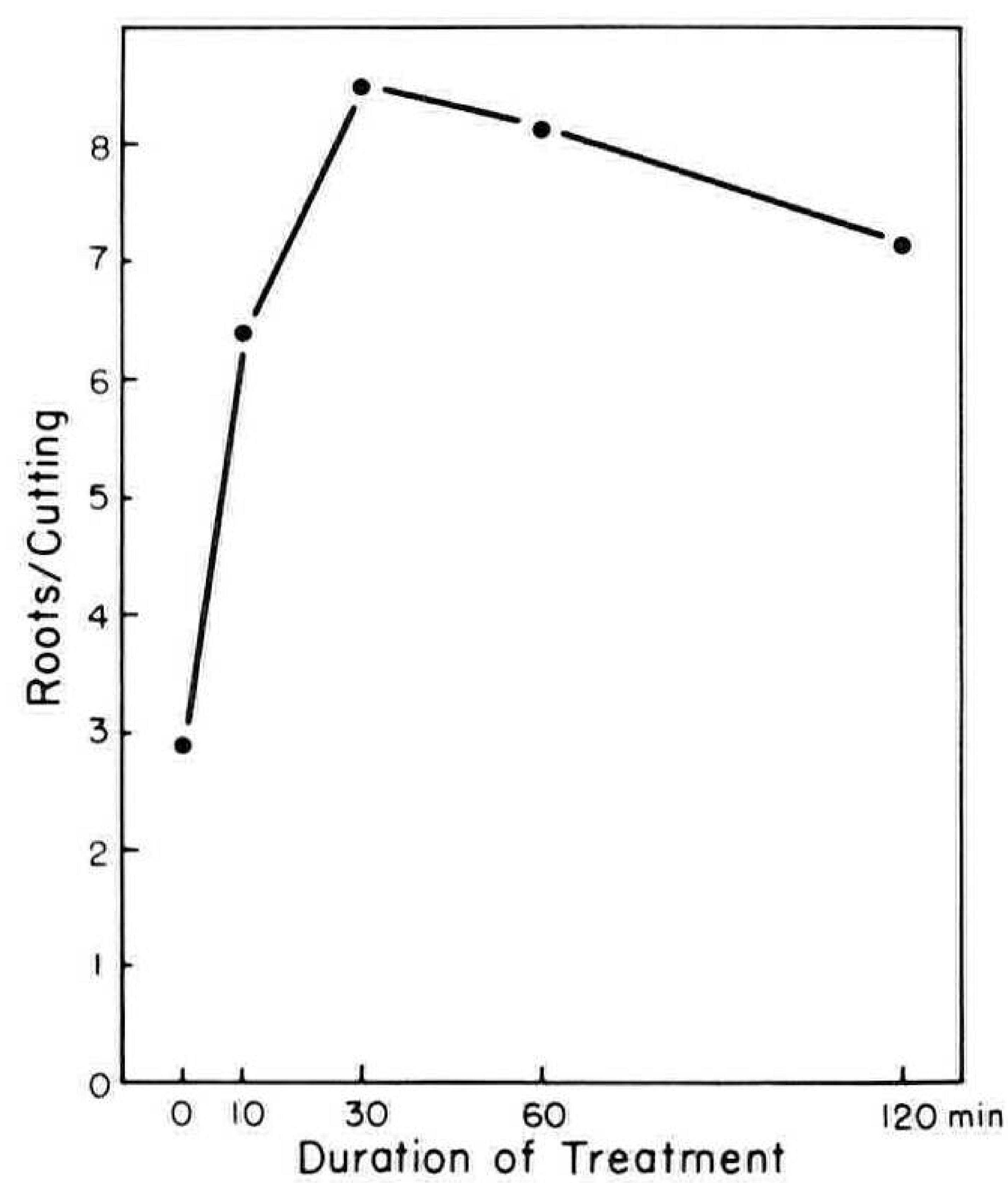

Fig. 1. **Effect of ethylene gas on root formation in *Salix fragilis* softwood cuttings. Cuttings were exposed to ethylene gas for 0, 10, 30, 60, or 120 min and then soaked upright in water 4 cm deep until root formation. (2).**

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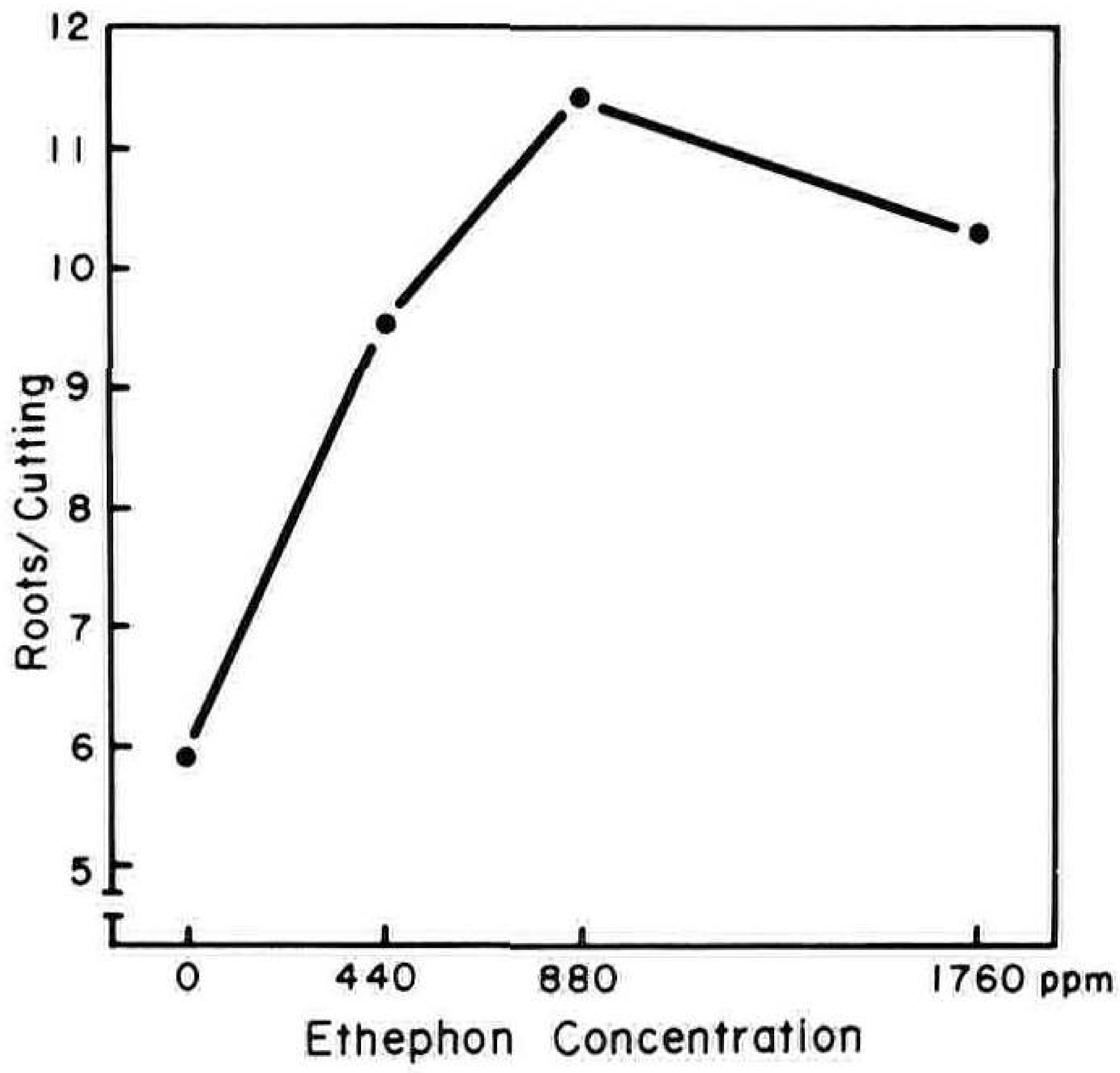


Fig. 2. **Effect of Ethephon on root formation in *Salix fragilis* softwood cuttings. The basal 4 cm of cuttings were soaked upright in 0, 440, 880, and 1760 ppm of Ethephon for 24 hr. then soaked in fresh water 4 cm deep until roots formed. (2).**

Fig. 3 **Effect of Ethephon on rooting of tomato cuttings. Bases of cuttings were placed in 400 ppm solution of Ethephon for 24 hr before sticking in propagation medium.**

Ethylene gas has been considered a plant growth regulator and it has, besides stimulation of root formation, many other morphogenic functions such as breaking of bud dormancy, causing leaf epinasty and leaf chlorosis, controlling stem growth, stimulating flower bud initiation, changing flower sexes, wilting of flowers, causing abscission of leaf, flower, and fruit, controlling seed germination, and controlling root growth.

Ethylene is synthesized by the plant itself. For example, it is well known that apple fruits should not be kept in storage with cut flowers. Ethylene gas evolved from the fruits causes carnation to sleep, induces leaf epinasty, and causes roses to shed petals. This is because apple fruits are a rich source of ethylene gas. According to our study (3), the internal gas of apple 'Red Gold' fruits contained over 120 ppm of ethylene. Ethylene is produced by many other parts of plants besides fruits, such as leaves, stems, roots, flowers, tubers, and seeds.

Ethylene production by plants can be examined easily by measuring the ethylene concentration of internal gas extracted from a specific plant part. For instance, when one wants to extract the internal gas of cuttings, cuttings are placed under an inverted funnel filled with gas-free water. The neck of the funnel is sealed with a serum cap. The funnel is placed inside a vacuum desiccator which is half filled with water. The desiccator is then evacuated for 5-30 min by a water aspirator. Under such conditions, internal gas bubbles out mostly from the cut ends of the cuttings and are collected inside the funnel (Fig. 4). A sample can be obtained easily from the collected gas with a syringe through the serum cap. In normal atmospheric pressure, internal gases, including ethylene, diffuse out of the cuttings through lenticels as well as through cut ends. Therefore, ethylene gas production by the cuttings also can be studied by sealing the cuttings in an airtight container. After a certain period of time, a small amount of gas is removed from the container and the ethylene concentration in the gas is measured. The gas chromatograph is a very useful tool for quantitative determination of very low concentrations of ethylene gas. For instance, the instrument can detect ethylene concentration as low as 5 parts per billion yet only 1/5 teaspoonful of gas is required for the test.

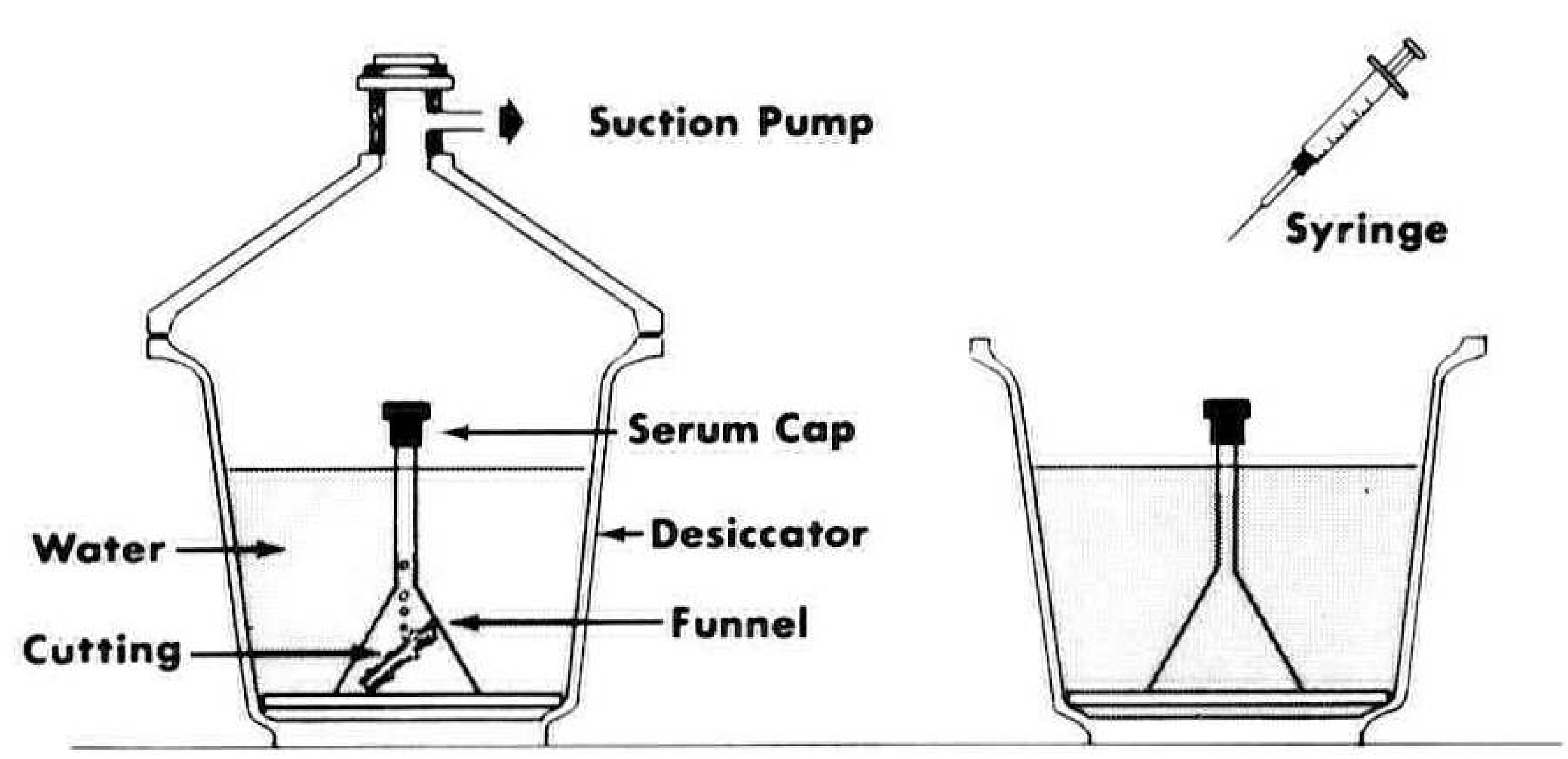


Fig. 4. Extraction method of internal gas from plant materials. Internal gas bubbles out of the plant materials under reduced pressure and is collected in the funnel (left). After removing the desiccator lid, the gas is sampled by syringe through the serum cap for gas analysis (right).

At the 1965 meeting of the International Plant Propagators Society in Cleveland, I presented a paper entitled, "Centrifugation promotes rooting of softwood cuttings" (1). According to that paper, when softwood cuttings of some woody species, including *Salix alba*, *S. acutifolia*, *S. pentandra*, *S. fragilis*, *Viburnum dentatum*, and

Populus alba, were centrifuged with water, the cuttings rooted better than non-centrifuged controls. The results suggested that centrifugal force apparently accelerated the downward transport of rooting substances in the cuttings, thus causing the accumulation of the substances at the basal ends of the cuttings, which in turn stimulated root formation.

Recently, this author found interesting results which imply that the centrifugal force stimulates rooting of softwood cuttings partially through changing the ethylene concentration in the cuttings (2). During centrifugation with water, water is forced into the cuttings and the water content of the cuttings increases. This increased water in the cuttings apparently provides a barrier for diffusion of ethylene gas out of the cuttings which in turn causes a high concentration of ethylene gas. Thus, the resulting high ethylene concentration stimulates root formation. Even when cuttings were centrifuged with the same force, the deeper the cuttings were steeped in the water in the centrifuge tube, the more water was forced into the cuttings thus resulting in more roots (Fig. 5).

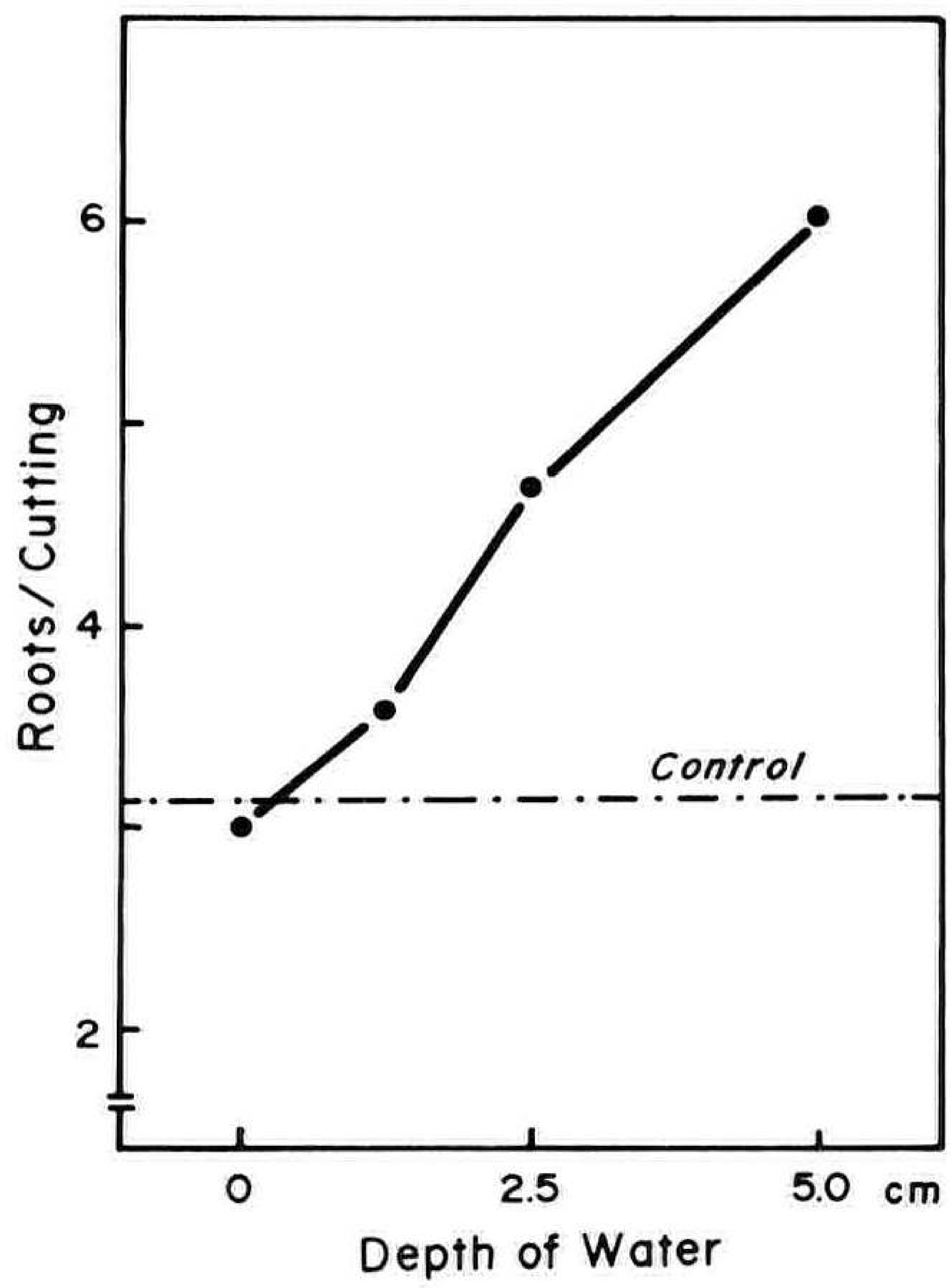


Fig. 5. Effect of water depth during centrifugation on root formation of *Salix fragilis* softwood cuttings. Except for non-centrifuged controls, cuttings 10 cm long were centrifuged for 1 hr at 2000 g with the basal 0, 1.25, 2.5, or 5 cm being soaked in water. After centrifugation, the basal 2.5 cm of all cuttings, including controls, were cut back and the resulting 7.5 cm portions were soaked in water 4 cm deep until root formation. Dotted line indicates number of roots produced by controls (2).

Even without increasing water content of the cuttings by centrifugal force, one can increase the internal ethylene concentration of the cuttings simply by keeping the cuttings in water. For instance, when cuttings of such plants as crabapple, chrysanthemum, and privet were completely submerged, ethylene concentration increased significantly within 20 hr (Table 1). Thus, submersion of cuttings resulted in better rooting, as we might expect (Fig. 6). The deeper the willow cuttings were steeped in water for 24 hr, the more

Table 1. Effect of submersion on ethylene concentrations in crabapple, chrysanthemum, and privet cuttings. Cuttings were completely submerged in water at 24° C for 20 hr and controls were steeped upright in water 2.5 cm deep. (3).

Cuttings and treatment	Ethylene concentration (ppm)			
	Before treatment	After treatment	LSD 5%	LSD 1%
Crabapple				
Control	0.07	0.32	0.13	0.23
Submerged	0.06	1.66		
Chrysanthemum				
Control	0.06	0.37	0.30	0.55
Submerged	0.06	0.67		
Privet				
Control	0.06	0.50	0.40	0.74
Submerged	0.06	1.20		

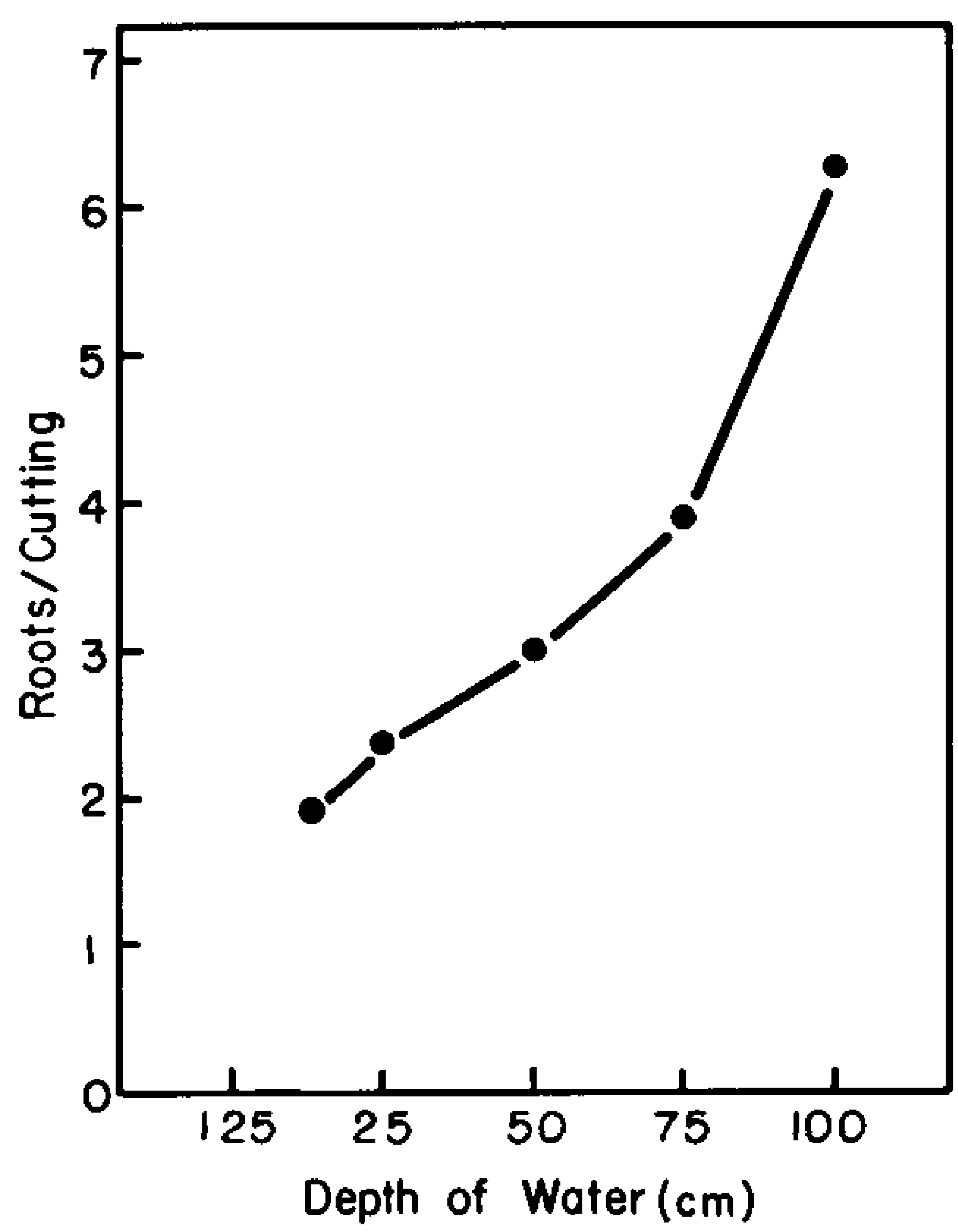
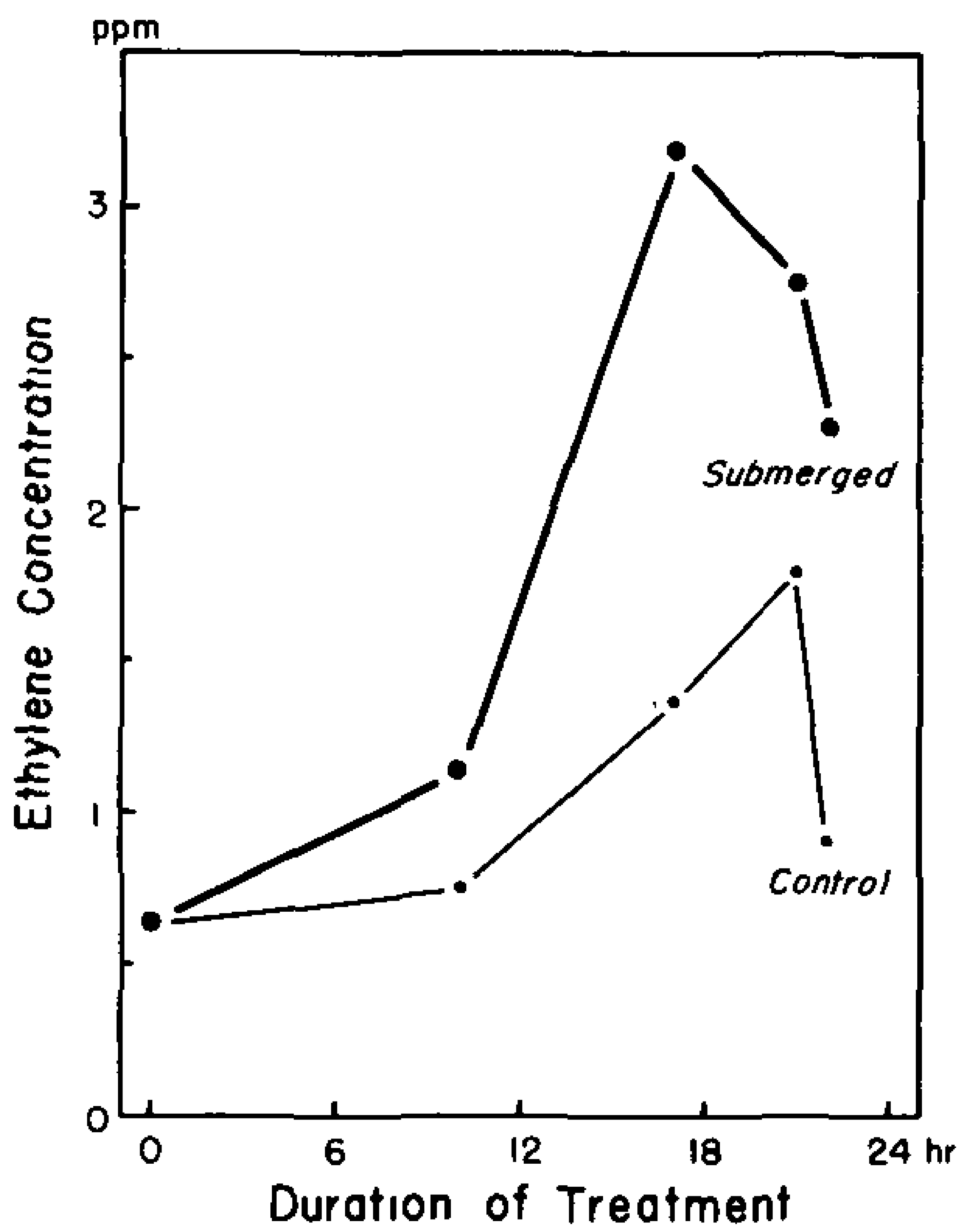
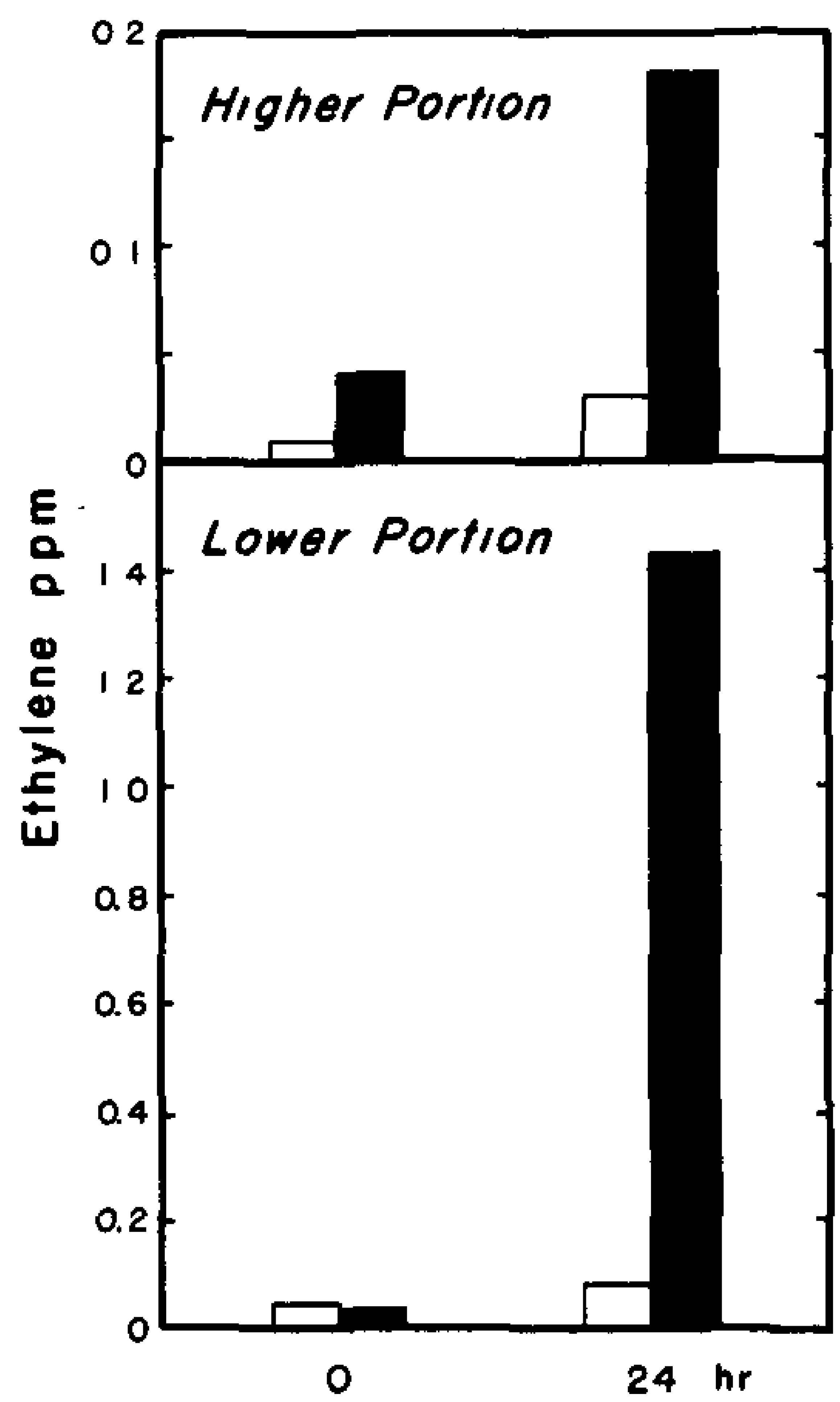


Fig. 6. Effect of soaking-water depth on root formation of *Salix fragilis* softwood cuttings. Cuttings 10 cm long were soaked upright for 24 hr in water 1.25, 2.5, 5.0, 7.5, or 10 cm deep and then transferred into water 4 cm deep until roots formed (4).



7. Effect of water soaking on ethylene concentration in *Salix fragilis* softwood cuttings. Cuttings were completely submerged upright in water, while in controls the base, 2.5 cm of cuttings were soaked upright in water. After treatment of 10, 17, 21, and 22 hr, gas samples were collected from cuttings and their ethylene concentrations were measured (2).



8. Effect of flooding on ethylene concentrations in sunflower plants. Plants were flooded up to midway between cotyledonary and 1st leaf node for 24 hr while controls were not flooded. Ethylene was extracted from lower portions (stems below 1st leaf node and roots) and high portions (stems between 1st and 3rd leaf nodes). White and black bars refer to controls and flood treatments.

roots formed from these cuttings. Soon after cuttings are submerged in water, residual oxygen trapped in the cuttings is exhausted by respiration. Since ethylene synthesis does not proceed without oxygen, ethylene concentration gradually declines after 20 hr in submerged cuttings (Fig. 7).

Quite often, root systems of intact plants are saturated with water Plants grown in containers with heavy soil mixtures often undergo such a condition. Overwatering worsens the situation. Flooding is a common natural occurrence and sometimes plants in the field are flooded after heavy rains. According to our research results, when the base of an intact plant is flooded, ethylene concentration starts to increase in the submerged portion of roots and stems. As seen in Fig. 8, ethylene concentration in the submerged portion of sunflower increased 48 times after 24 hr flooding. There was an apparent gas exchange between submerged and non-submerged portions because ethylene concentration gradually increased in higher parts of the plants which had not been submerged.

Flooding, which costs us millions of dollars annually, even when limited to agricultural commodities, causes different kinds of damage to crops depending on the depth and duration of the flood, age and height of plants, and environmental factors such as temperature, wind velocity, sun's radiation energy , and so on. Generally speaking, flooding damage symptoms are (A) wilted leaves, (B) epinasty, (C) leaf chlorosis, (D) reduced stem elongation, (E) enlarged stem diameter, (F) formation of new adventitious roots. It is very interesting to note that all of these symptoms can also be caused by ethylene gas. It is believed that increased ethylene concentration in flooded plants is largely although not exclusively responsible for the flooding damage symptoms (4).

LITERATURE CITED

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RALPH SHUGERT: Thank you, Mak, for an interesting and detailed paper on a fascinating topic.

The next paper on your program has been selected for the graduate student award this year. The author, David Hamilton of Purdue University, could not be with us so his paper will be read by Dr. Phil Carpenter, also of Purdue.