CENTRIFUGATION PROMOTES ROOTING OF SOFTWOOD CUTTINGS[1]

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According to Cooper (1) and Went (4), the polar transport of auxin causes it to accumulate at the base of a cutting and the resulting auxin gradient causes a downward movement of rhizocaline towards the base of the cutting. Rhizocaline and auxin, thus accumulated at the base of the cutting, act together in root formation. This classical hypothesis suggested a search for some means of modifying the polar transport of auxin and rhizocaline in cuttings. Centrifugal force was applied to willow cuttings (*Salix alba* L.) which are easy rooters and it was found that centrifugation promotes rooting in this species. Although this new finding has already been published (2, 3), I would like to briefly review my work for you including results of a preliminary nature.

Centrifugation and Rooting

Leafless or leafy softwood cuttings were placed in a centrifuge tube with distilled water and centrifuged using an ordinary laboratory centrifuge. Centrifugal force was directed from the tip to the base of the cutting. The root-promoting effect of centrifugation increased not only depending on the increase of cen-

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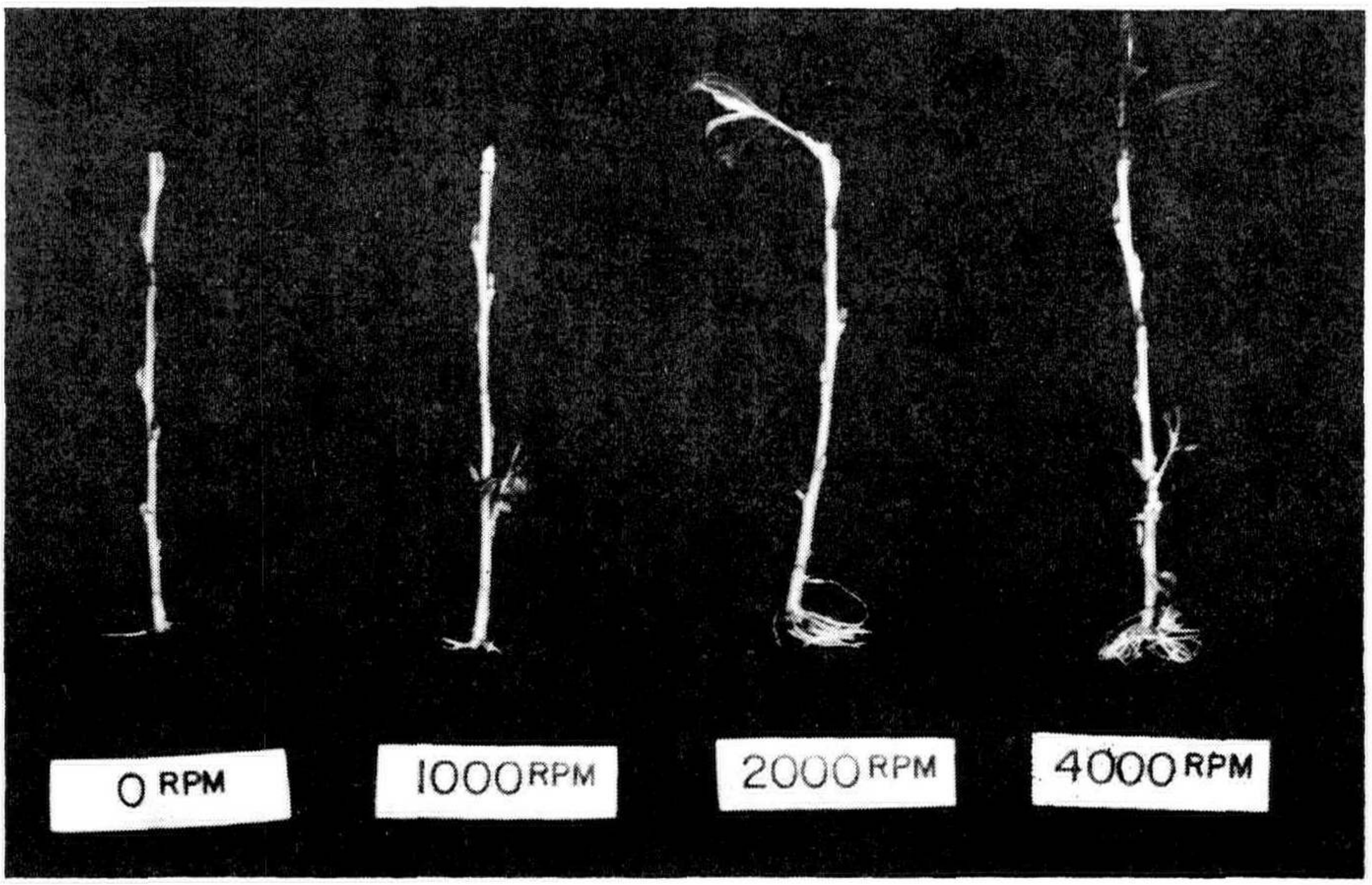

Figure 1. Effect of centrifugation on rooting of willow cuttings. Cuttings were centrifuged for one hour with speeds of 0, 1000, 2,000 and 4000 rpm or with gravitational forces of 0, 160, 640 and 2540 g.

trifugal force but also depending on the duration of centrifugation (Fig. 1 and Fig. 2).

Physiological Implication of Centrifugation

It was, however, found that the more the proximal ends were cut back after the centrifugation, the fewer the roots that were formed (Fig. 3). Willow cuttings were next centrifuged in the up-side-down position, i.e., the force was directed from the base to the tip of the cuttings. By this up-side-down method, rooting of the cuttings declined proportionally to the increased duration of centrifugation (Fig. 4).

What these results suggest is that some root-promoting substance was forced to move down towards the direction of the force. This idea was emphasized by a new finding. As mentioned earlier, cuttings were centrifuged in a centrifuge tube with distilled water. When the water or diffusate was tested after the centrifugation by mung bean rooting test, it showed a strong root-promoting effect. As seen in Fig. 5, root formation of centrifuged willow cuttings increased proportionally to the increasing gravitational force over the range between 0 to 2540 *g*. It is, however, most interesting that the root forming activity in the centrifugal diffusate as tested in the rooting of

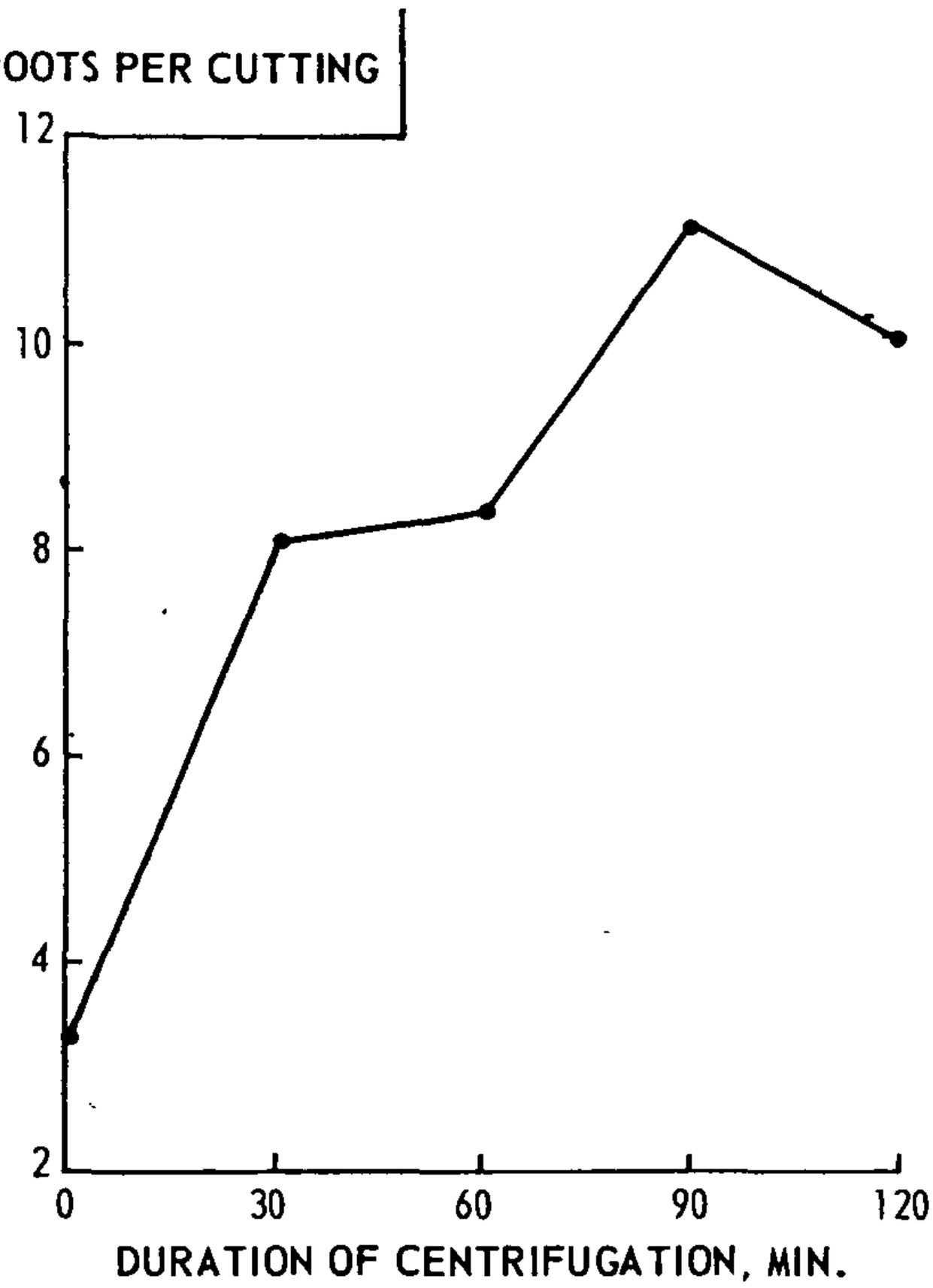

Figure 2. Duration of centrifugation and rooting in willow cuttings. Cuttings were centrifuged with a force of 640 *g* (Kawase, 1964).

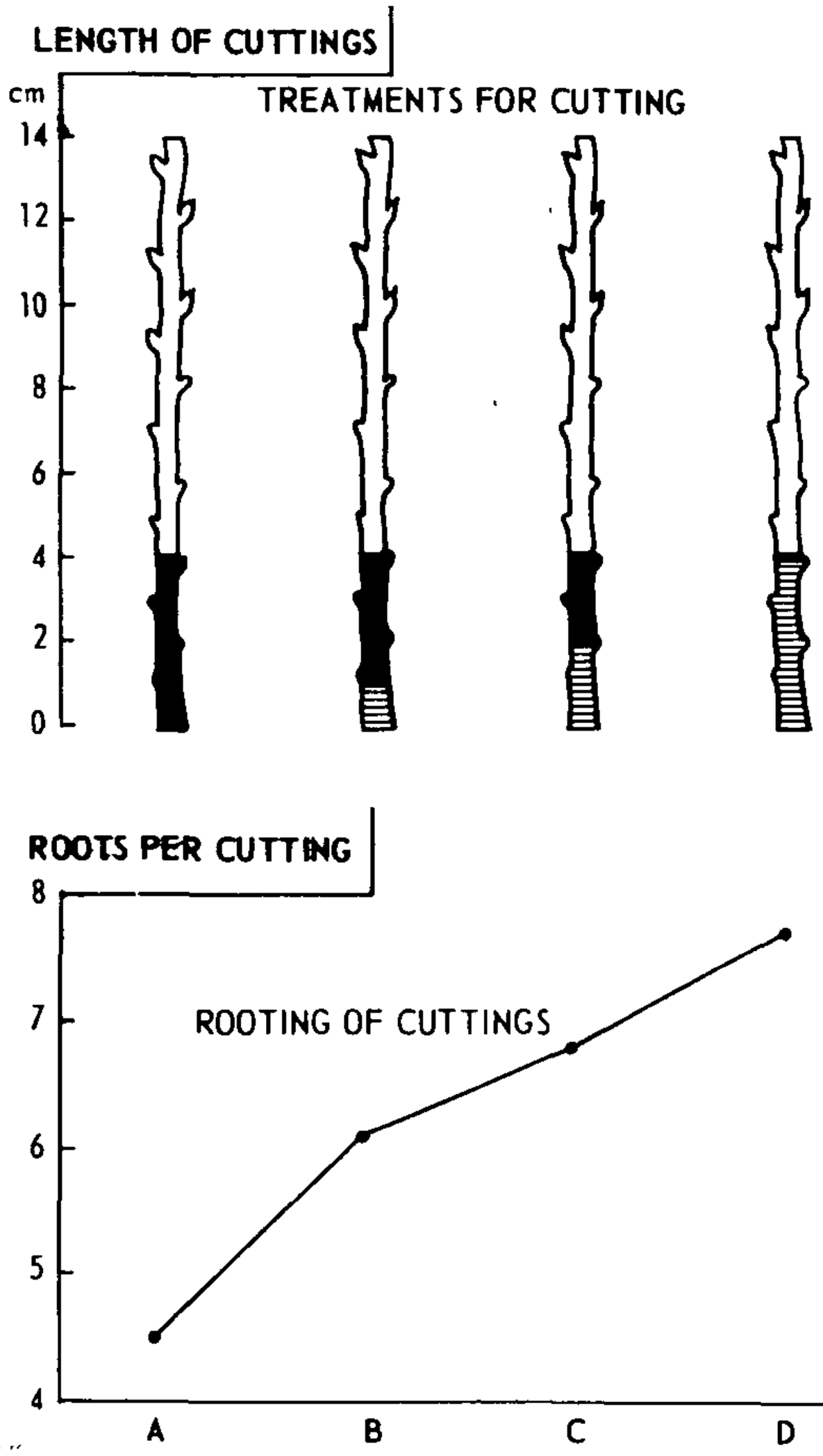

Figure 3 Effect of cutback on the rooting of centrifuged cuttings Cuttings were centrifuged for 1 hour with a force of 1090 *g* Shadowy and black parts of the cuttings represent parts which were cut back before and after the centrifugation (Drawn from Kawase, 1964).

mung bean cuttings also increased with the greater force of centrifugation within the range between 0 to 1940 *g*. The results clearly suggested that there was an accumulation of root-forming substance (or substances) at the proximal ends by the centrifugation and also that a part of it diffused into water.

Rooting Substances in Centrifugal Diffusate

Paper chromatographic study of the diffusate revealed that because of its immovability on the paper chromatograph with 80% isopropanol, the major root-promoting substance in the diffusate is not indoleacetic acid (Fig. 6). Paper chromatographic study also showed that the major root-promoting substance in the diffusate promoted mung bean rooting by itself

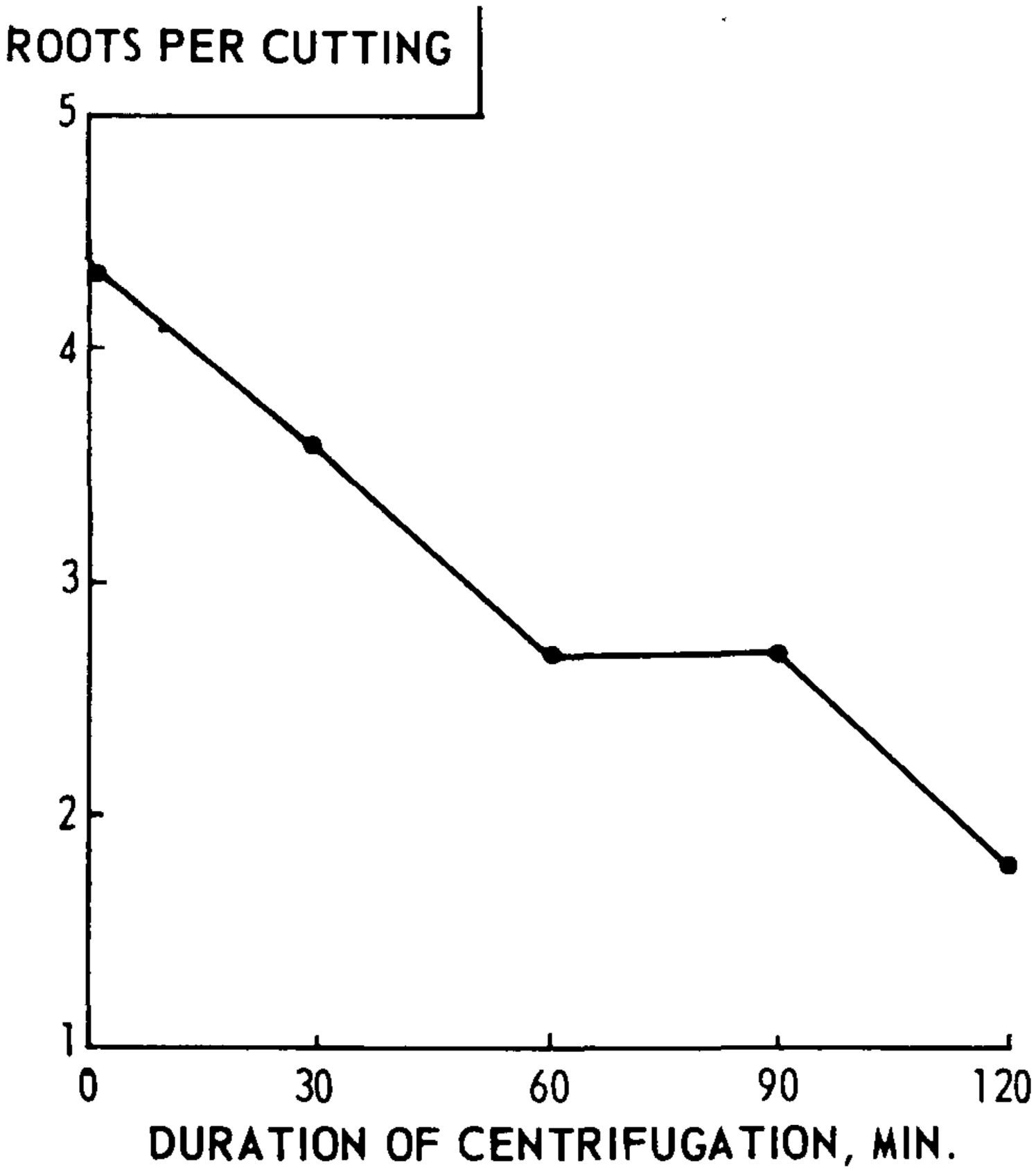

Figure 4 Effect of up-side-down centrifugation of willow cuttings on their rooting Centrifugal force of 640 g was directed from the base to the tip of the cuttings (Kawase, 1964)

and its effect was enhanced by the presence of indoleacetic acid (Fig. 6 and Fig. 7).

On the other hand, centrifuged cuttings which are supposed to contain a high level of the same rooting substance as seen in the diffusate, showed an interesting result when treated with indoleacetic acid. As seen in Fig. 8, the root-promoting effect of centrifugation was enhanced by the presence of indoleacetic acid. Rooting of centrifuged cuttings was also enhanced by etiolation treatment (Fig. 9). Etiolation treatment for the cuttings was recently found to delay the destruction or disappearance of indoleacetic acid in the cuttings (3).

It is noteworthy here that the diffusion method is one of ideal methods to extract rooting substances from cuttings because it enables one to extract the substances in the form in which they exist in the living plant tissue without any chemical treatment.

Conclusively, the rooting substance (or substances) in the diffusate is thought to be similar to rhizocaline, and it is proposed that centrifugation accelerates the transport of rhizocaline and thus the accumulation of rhizocaline at the proximal ends.

Future Application

Centrifugal root promotion is not limited to the specific species, *Salix alba* L. As far as I have studied, centrifugation promoted rooting of softwood cuttings in *Salix acutifolia, S. pentandra, S. fragilis, Viburnum dentatum* and *Populus alba.* More studies are needed before we find out how widely this centrifugation method can be recommended to the nursery industry. One point which has to be mentioned is that the centrifugation process can damage the leaves of cuttings. Thus a root-inhibitory effect instead of root-promoting effect was found in centrifuged *Philadelphus* and *Cornus* cuttings. My preliminary

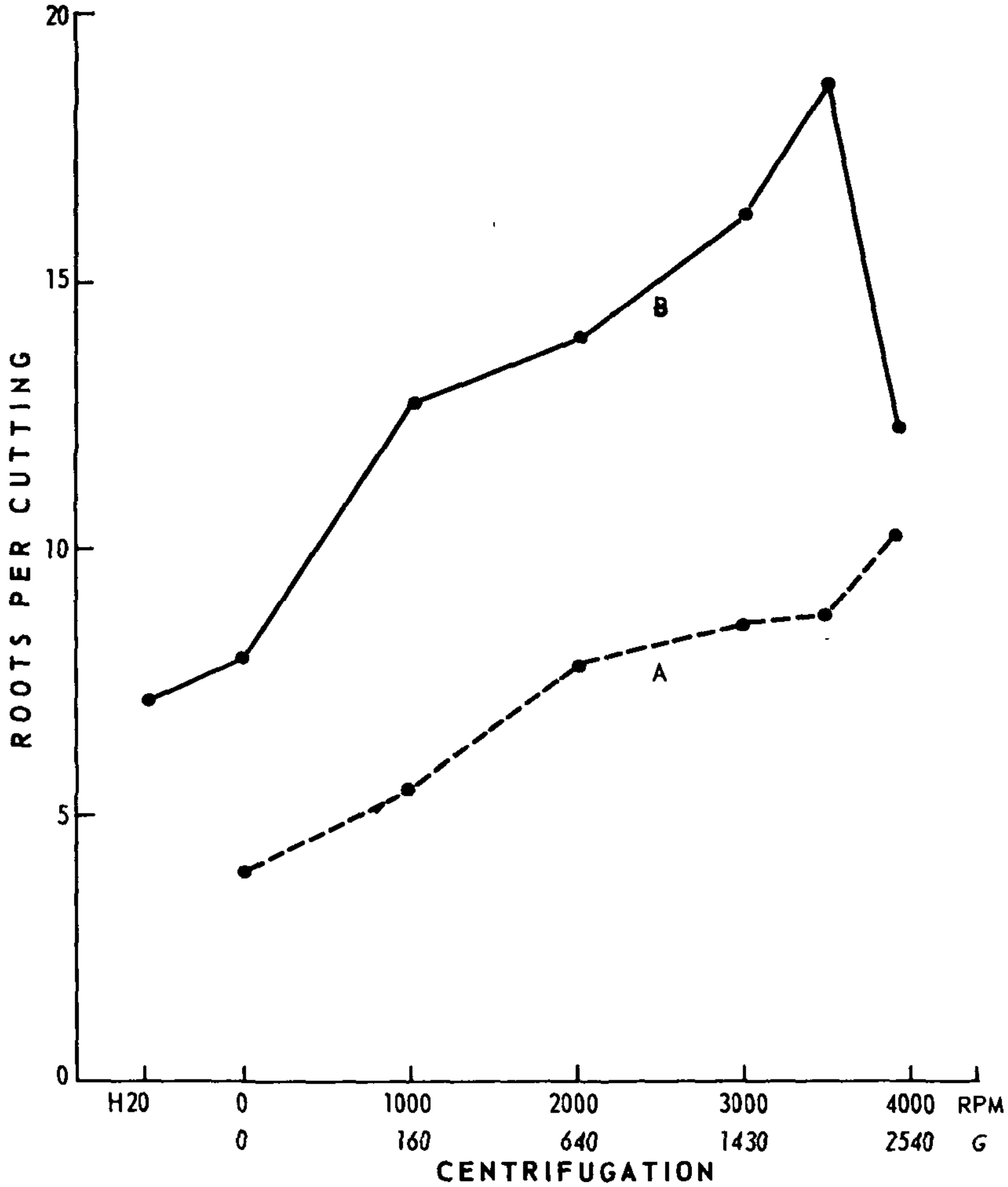

Figure 5. Effect of centrifugation on willow rooting (A), and the effect of the diffusate on mung bean rooting (B) Willow cuttings were centrifuged and their rooting was observed in distilled water (lower), while their centrifugal diffusates were assayed by mung bean cuttings (upper) (Kawase, 1964)

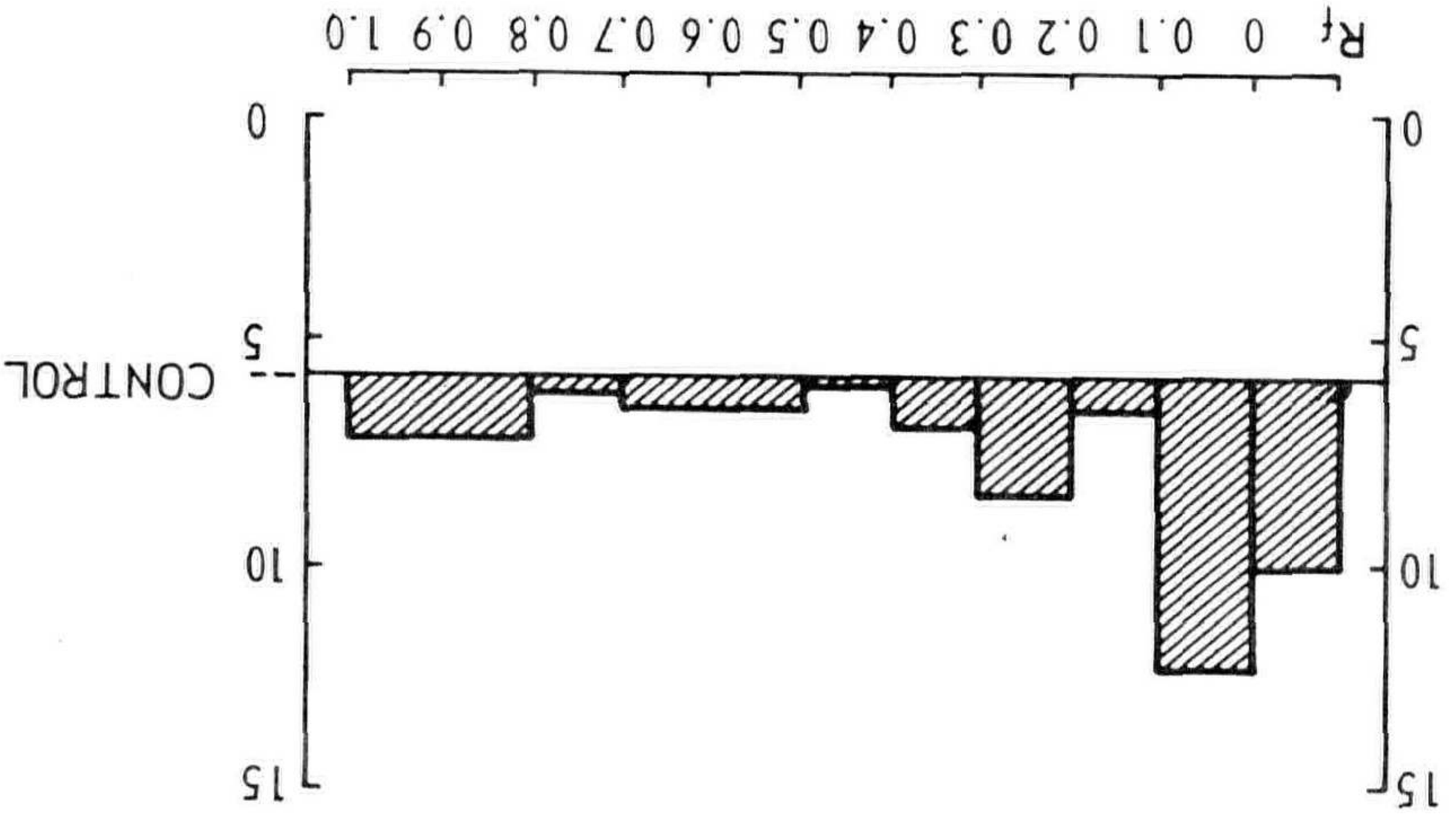

Figure 6. Histogram indicating the rooting substances of the centrifugal diffusate from 60 willow cuttings. Abscissa: Rf values in 80% isopropanol (v/v). Ordinate: No. of roots per mung bean cutting (Kawase, 1964)

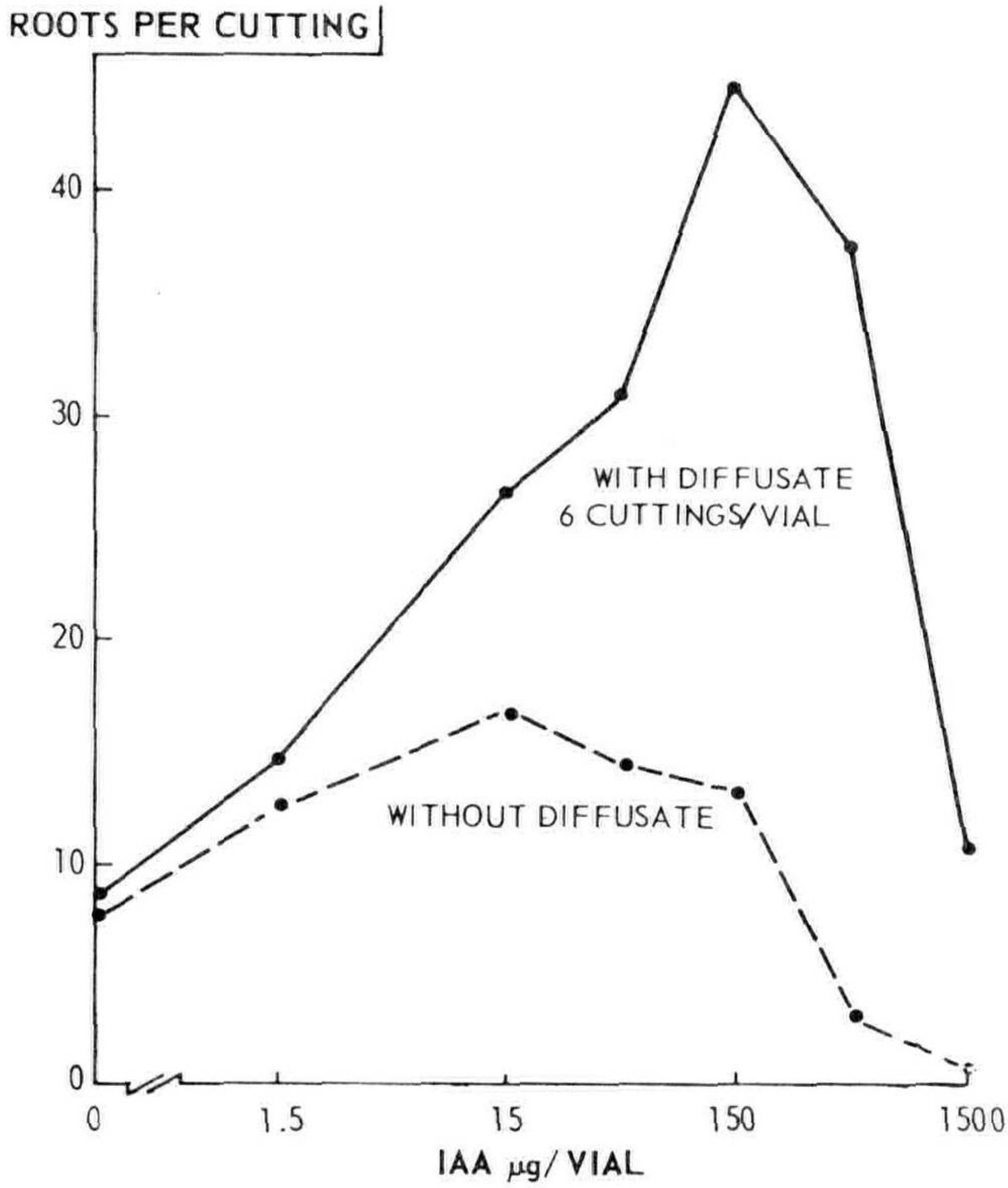

Figure 7. Synergistic effect of centrifugal diffusate and IAA on the rooting of mung bean cuttings (Kawase, 1964).

results show that many easy-to-root species apparently contain diffusible rooting substance(s). The root substance was shown to be effective also in cuttings such as *Euonymus obovata, Syringa pubescens* and *Cotoneaster lucida.* Therefore when more is known about the chemical nature of the substance, there will be a great interest in its future use to promote rooting.

REFERENCES

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4 Went, F W 1938 Specific factors other than auxin affecting growth and root formations Plant Physiol 13 55-80

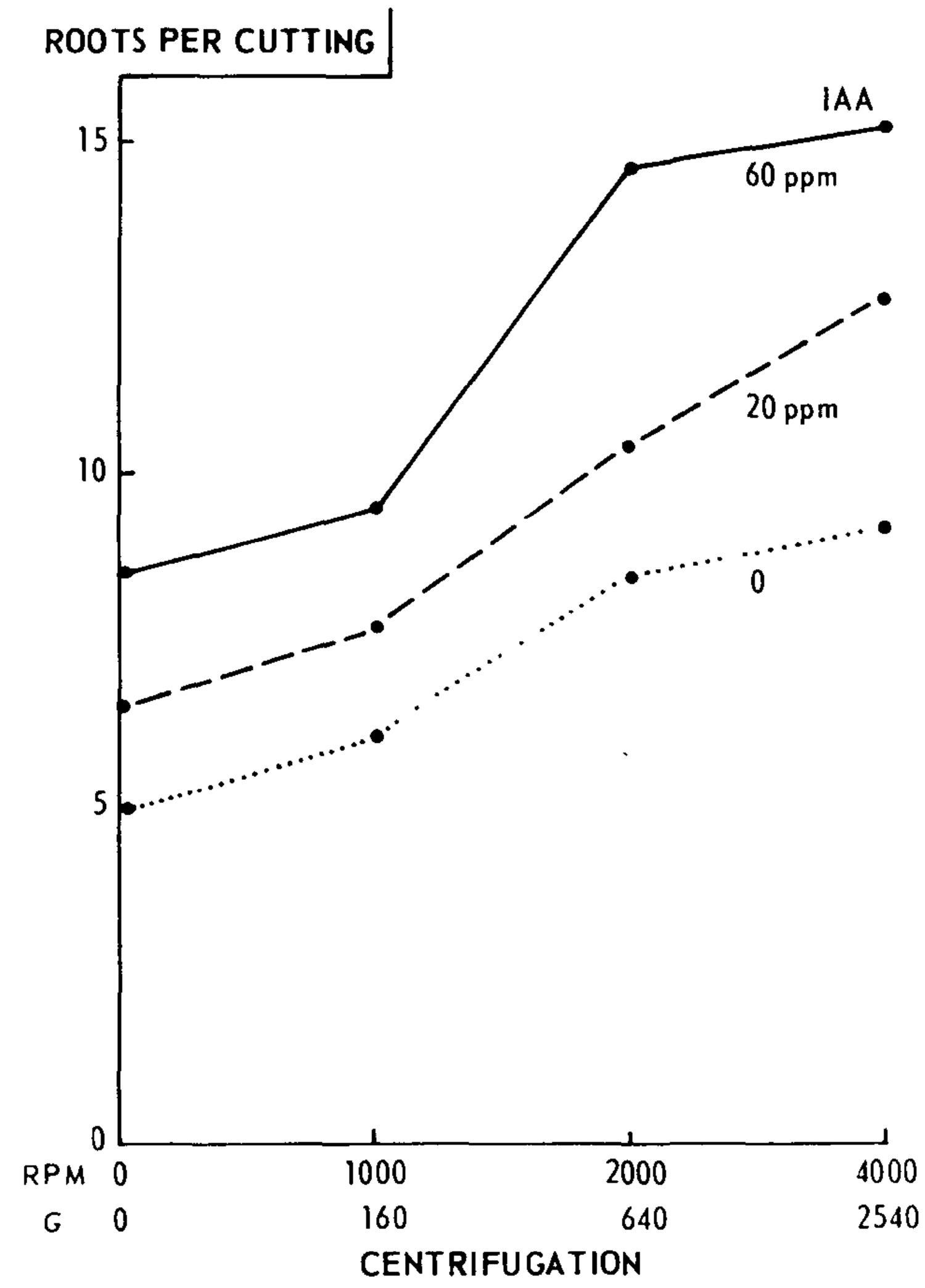

Figure 8 Combined effect of centrifugation and IAA on willow rooting After centrifugation willow cuttings were treated by IAA (Kawase, 1965).

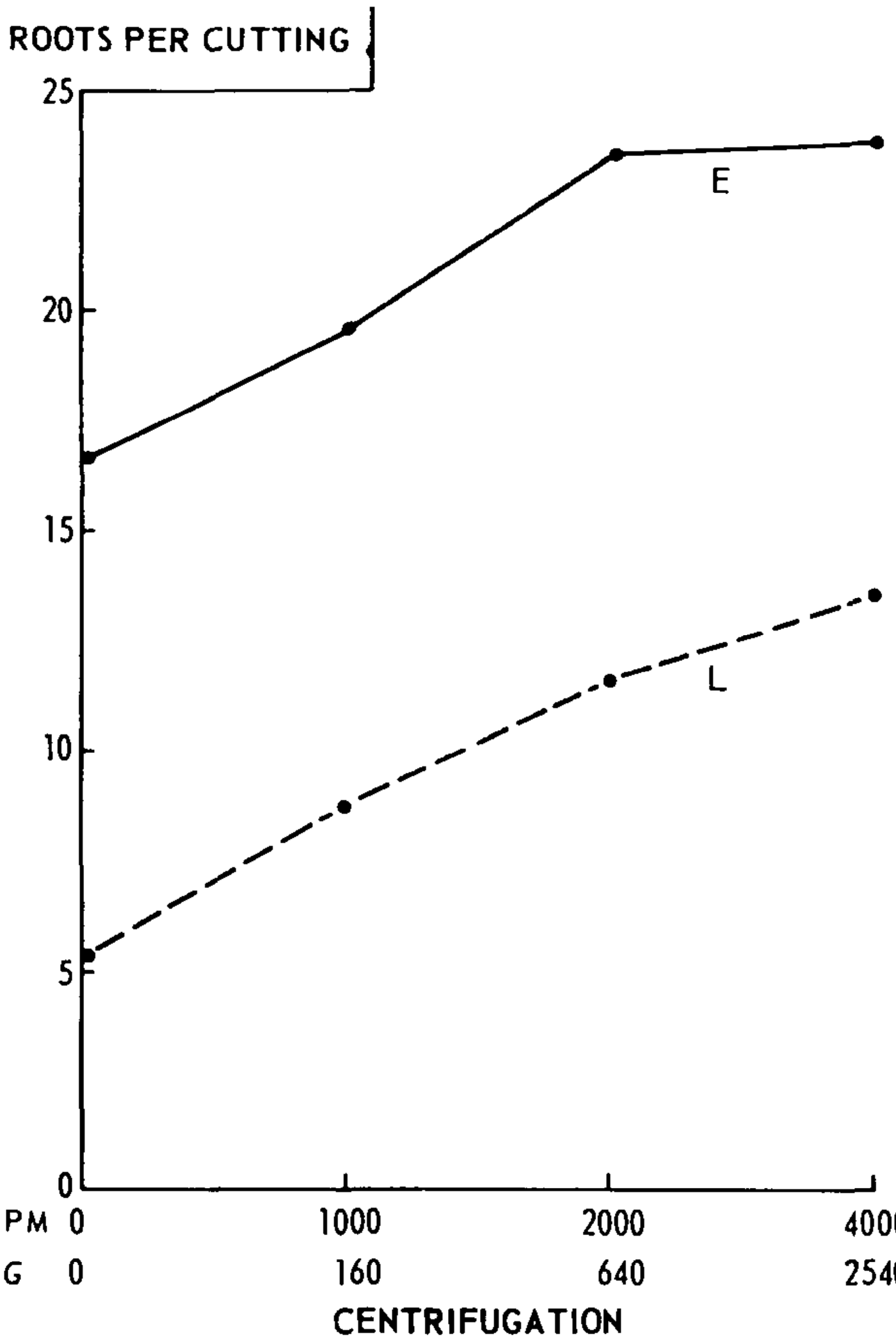

Figure 9 Combined effect of centrifugation and etiolation on rooting of willow cuttings. Centrifuged cuttings were etiolated (E) or not etiolated (L) (Kawase, 1965).

HOWARD STENSSON: Dr. Kawase, I just wondered if you tried the diffusate from the tips on the bases of the cuttings.

DR. KAWASE: I have tried upside down diffusion and it is as concentrated as the base. However, root formation at the base is retarded when the cuttings are centrifuged upside down.

HOWARD STENSSON: And a second question. Did you try the diffusate from one species on another species?

DR. KAWASE: Most species I have tested on the mung bean cutting test. I have extracted from willow and then retreated willow. It was most effective on the cuttings not previously centrifuged.

TREVOR SYKES: It's been known for a long time that willows root very easily or many of the willow species do because of the presence of primordial root initials. Have you found or

examined the cuttings for any of these root initials?

DR. KAWASE: I cannot answer the question directly. However, I can say that the diffusate did promote rooting in mung bean cuttings as well as increase rooting in the willow cuttings. The mung bean cuttings have no performed root initials. So I know that it will promote root initiation as well as development of the initials.

VOICE: Did you observe any vascular cell tissue damage on your herbaceous material at 4000 rpm?

DR. KAWASE: Yes, this is sometimes a problem even with willow at the higher speeds.

DR. HESS: We could add another species here, Mokato, *Hedra helix* also works except that we get both 1 and 4 cofactors out from the diffusate.

DR. KAWASE: Yes, I understand that was reported in the last Proceedings.

DR. HESS: That was just straight diffusion but we've also used your centrifugation technique.

DR. KAWASE: Oh, you have used it.

DR. HESS: Yes, I think centrifugation is better because with diffusion over a period of time you have bacterial contamination, but by centrifuging just for an hour I think you get relatively pure materials without contamination.

MODERATOR PINNEY: The next subject, propagation of *Picea pungens glauca* will be given by Mr. Leonard Savella.

PROPAGATION OF PICEA PUNGENS GLAUCA CULTIVARS

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The propagation of Blue Spruce from either seed, layering or grafting has been a practice for many years. It was believed that these were the only methods to reproduce this species commercially and make it profitable.

With the introduction of the mist system of propagation, the Spruces, like many of the other ornamentals that were reproduced in one of these three ways, have become easy to root from cuttings. The procedure is very much the same as most of the ornamentals propagated under mist, except that just a little more care should be taken in timing your cutting.

The following is a step by step method that we use at Bald Hill Nurseries, Inc. which has proven to be very successful:

An outdoor mist bed 6′ wide is prepared with a layer of peat moss 1½ - 2″ thick. Then a layer of sharp sand 6″ thick is put on top of the peat moss. The sand is then rolled with a roller filled with water for compaction. The sand is leveled off so that the surface area is as level as possible. The mist pipes are then placed on top of the bed and connected. Our system is