time to produce roots before the onset of cold weather. If so the cold of winter would condition the shoot-buds and shoots would develop in spring. Those ripening late in the season, however, would be categorized as two-year seeds. Their roots would not develop until the following summer, shoot-bud dormancy would be overcome the second winter and the completed plant would grow during the next spring.

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MODERATOR SHUGERT: Thank you, Al; a great paper. Our next speaker is Harold Pellett from the University of Minnesota. Harold and I first met in Nebraska several years ago where we both enjoyed some interesting propagation challenges. Harold's paper covers the puzzle of "Seed Stratification".

SEED STRATIFICATION

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The term stratification was coined as a description of the common means of handling seeds during the period in which their dormancy conditions were satisfied. This is the practice of alternately placing layers of seeds between layers of moist sand or peat or other suitable media. The term is used today to describe all methods of storing seeds in a moist condition such as mixing the seed with the medium or fall sowing directly in the seed bed. When we think of stratification we commonly think of cold stratification to satisfy some internal dormancy problem, but storage of seeds in a moist medium under warm temperatures is also quite helpful in overcoming some types of dormancy, so we should say warm or cold to preface the word stratification depending on the conditions desired. A warm stratification period preceding a cold stratification is very helpful with some seeds

that have a double dormancy such as *Tilia americana*, those with immature embryo's such as *Fraxinus nigra*, and those with epicotyl dormancy such as some of the *Viburnum* species.

OPTIMUM CONDITIONS FOR STRATIFICATION

There are four environmental factors that are required to satisfy the after-ripening requirements of certain seeds. These are moisture, low temperature, adequate aeration and a certain amount of time. These are described in considerable detail in the 1960 proceedings by Kester (8). in general, 32° to 41°F is optimum for cold stratification for most materials (3, 20) and a diurnal temperature range of 68 to 86°F (20) is best for warm stratification. The exact moisture level does not seem to be critical (12, 20). The length of time required varies greatly from one plant to the next (20).

PURPOSE OF STRATIFICATION

Stratification is used in the broad sense to overcome some of the various dormancy conditions that were described in the previous paper by Fordham. Warm stratification is sometimes useful to speed up the physical breakdown of a hard seed coat or perhaps an exterior pericarp that delays water imbibition and gaseous exchange. The moist medium and warm temperatures are conducive to bacterial action that speeds the decay of the exterior covering or seedcoat. Cold stratification conditions are necessary for the seeds to undergo certain metabolic conditions that are necessary to overcome internal dormancy often referred to as after-ripening.

Cold stratification often results in a faster rate of germination as well as in a higher percent germination. Cold stratified seeds also frequently will germinate at lower temperatures than non-stratified seeds (1).

There have been several excellent papers given at previous IPPS meetings — by Kester in 1960 (8), Reisch in 1962 (15), and Allen in 1967 (1), covering the topic of stratification. I would also like to refer to two other papers. These are the review by Amen, "A Model of Seed Dormancy" (2), and the review by Wareing and Saunders, "Hormones and Dormancy" (22).

There has been considerable work by plant physiologists in an attempt to gain a better understanding of the processes involved during after-ripening of seeds. Since the endosperm and cotyledons of seeds store high quantities of foods in the form of fatty acids and other large molecules that don't readily release energy in simple metabolic processes, some of the earlier workers studied under the hypothesis that the after-ripening process re-

sulted in an increase in enzymes necessary for the metabolism of these fatty materials and a subsequent metabolism of these substances to simple sugars and other more readily available sources of energy. Kao and Rowan (9) found an accumulation of citrate, pyruvate, hexose monophosphate, sucrose and high energy phosphate during cold stratification of *Pinus radiata*. However the concentration of fatty acids was not significantly reduced. The concentration of the enzymes, lipase and invertase did not increase during cold stratification.

Barton and Bray (4) found an increase in concentration of some of the free amino acids during cold stratification of apple and tree peony seeds. However exogenous application of these materials failed to stimulate germination of dormant seeds.

With the improvement of techniques for identifying and quantifying levels of endogenous growth regulators there has been considerable activity in studies of the role played by these substances in regulation of seed dormancy. These studies have been quite rewarding and have shown a definite involvement of plant hormones in the regulation of internal dormancy. In many of these studies, inhibitors were found to be involved in the control of dormancy. During the cold stratification process, levels of these inhibitors decline (6, 11, 16, 23). These inhibitors can be found in either the embryo (23), the seed coat (16) or both (6). In other studies, growth promoting substances were found to be lacking or in low concentrations in dormant seeds, and were found to increase during the stratification process (11, 16, 21). These substances are commonly identified as one of the gibberellins, but the cytokinins (6, 11, 22, 24) and ethylene (7, 22) have also been found to promote germination. Although these studies have implicated the involvement of inhibitors and promoters there doesn't seem to be a simple control of the germination process by one hormone. Often the maximum level of a growth promoter is reached in a much shorter period of cold stratification than results in maximum germination (21, 24) or the level of an inhibitor is at the lowest point before maximum germination occurs (6, 11) 18). There appears to be an intricate balance of these growth regulators necessary before germination can occur. Inhibitors have been shown to prevent or retard the synthesis of gibberellins (17, 19). Perhaps the best piece of literature available on the subject is that written by Khan (10). He proposes a scheme, supported by research results, that shows that gibberellins are necessary for germination to occur. However, germination can be stopped by presence of inhibitors. A third group of hormones, the cytokinins can overcome the effect or counterbalance the inhibitors and enable germination to progress, but only if gibberellins are present (Figure 1).

	GIBBERELLIN	CYTOKININ	INHIBITOR	GERMINATION OR DORMANCY
1	+	+	+	G
2	+	+	−	G
3	+	−	+	D
4	+	−	−	G
5	−	−	−	D
6	−	−	+	D
7	−	+	−	D
8	−	+	+	D

Figure 1. Scheme proposed by Khan (10) to show hormone conditions that regulate internal seed dormancy

Although Khan's work was mostly with lettuce and some of the grasses and small grain crops that have a less intricate dormancy problem, his scheme could very well explain some of the results obtained in studies with seeds of some of the woody plants. Paul, et al. (14) and Biswas, et al. (5) working with loblolly pine seed found no or very little GA-like substances after 0 to 28 days of cold stratification and a high inhibitor level during the same period. After 42 days of cold stratification the promoter concentration increased gradually while the inhibitor level fell to almost zero. Exogenous applications of either GA_3 (100 mg/l) or kinetin (10 mg/l) resulted in good germination after 21 days of cold stratification, but GA_3 applications to non-stratified seeds only slightly promoted germination and kinetin gave no response to non-stratified seed. Diaz and Martin (6) working with peach found similar results. They found a synergistic effect of gibberellin and benzyl adenine, a cytokinin, in promoting germination but only after some cold stratification (Figure 2). They also found a rapid reduction in inhibitor (ABA) during the first two weeks of stratification while maximum germination required 12 weeks of cold stratification (Figure 3). In comparing a cultivar requiring a long stratification period to one with a shorter requirement they found more of the inhibitor in the cultivar requiring the longer period of cold stratification.

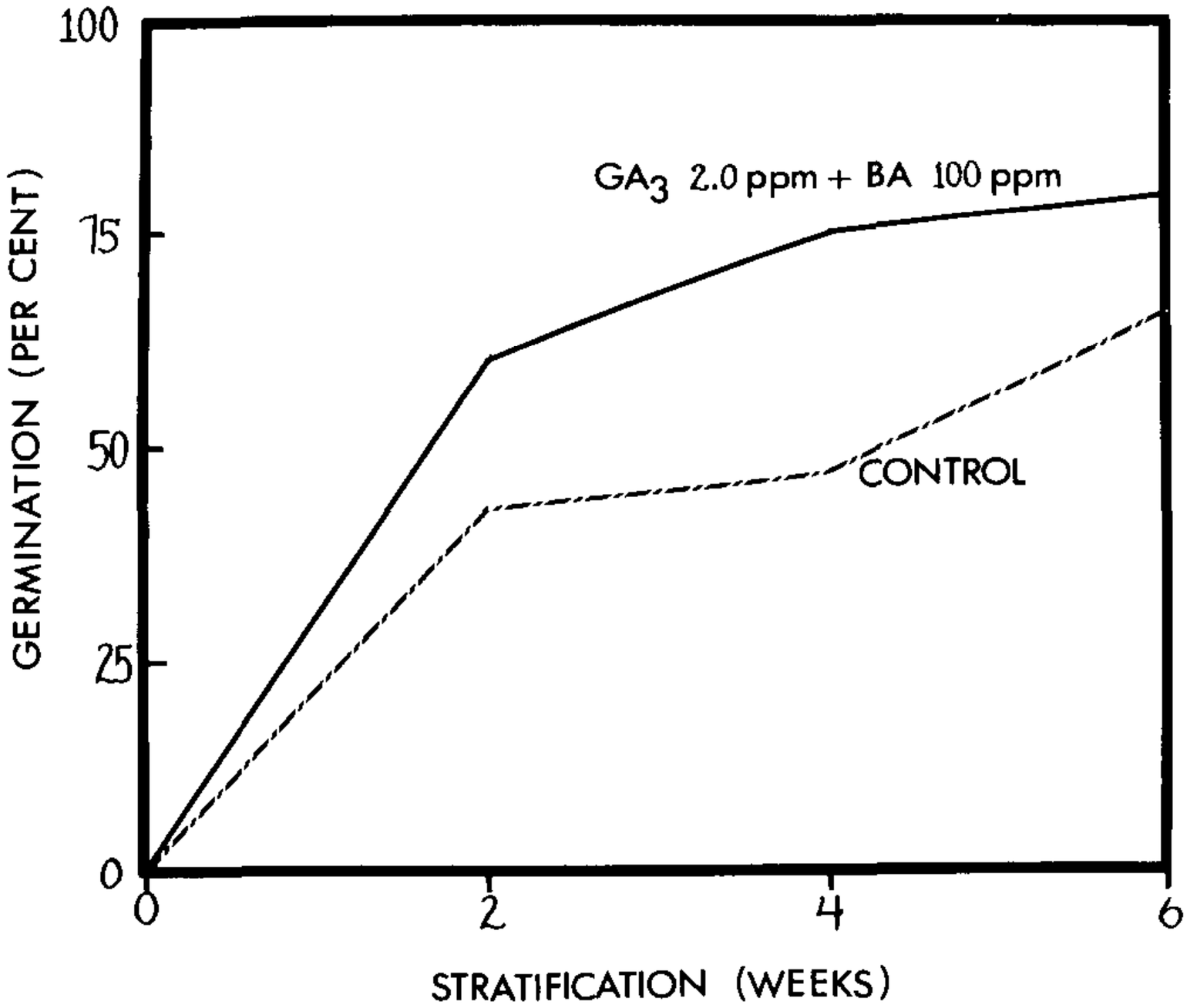

Figure 2. Effect of GA_3 and BA in stimulation of peach seed germination. From Diaz and Martin (6)

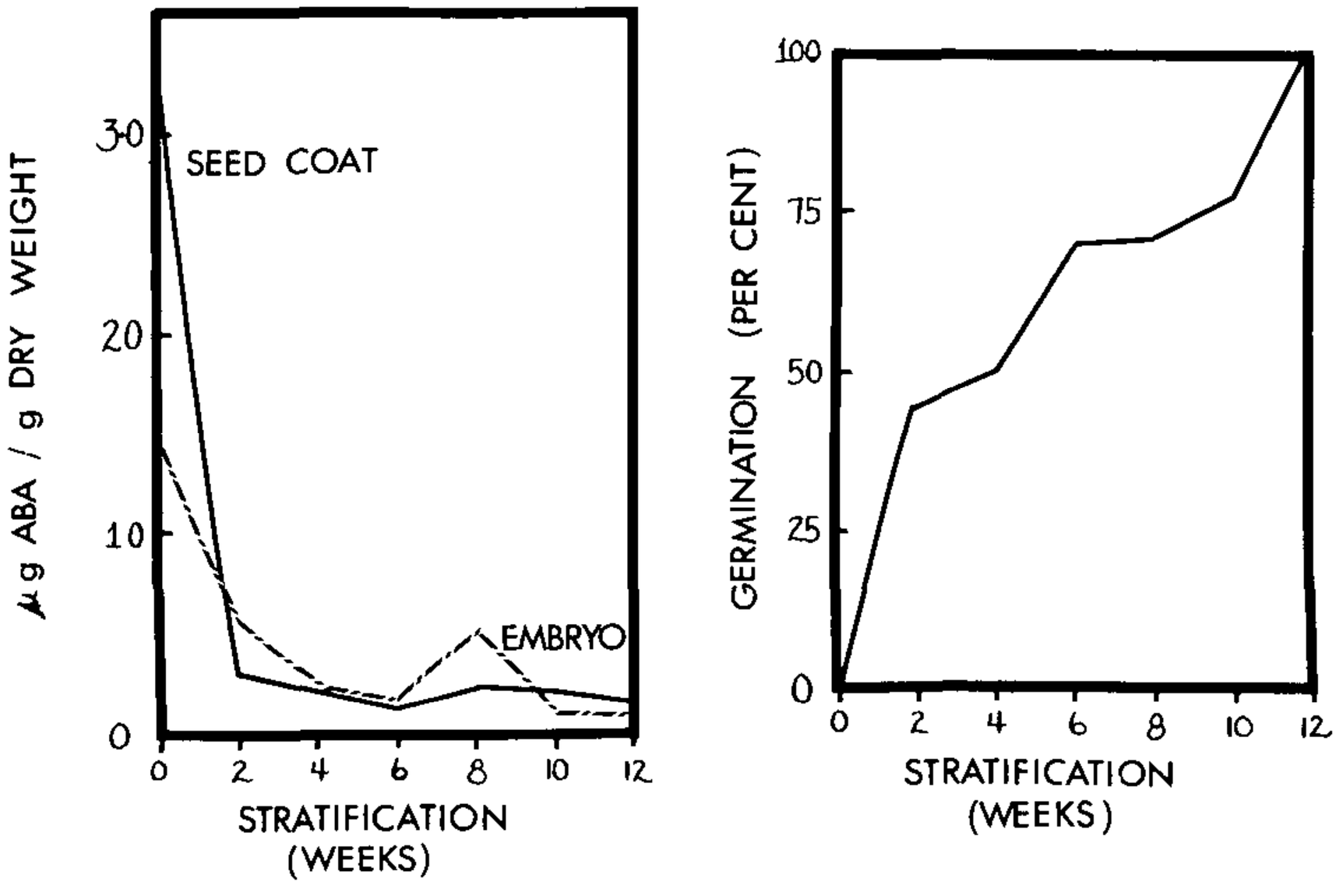

Figure 3. Inhibitor content and percent germination of peach seed after cold stratification of various time periods From Diaz and Martin (6)

Lin and Boe (11) working with plum seeds also found a rapid drop in inhibitor concentration (ABA) leveling off after 30 days of cold stratification, while a promoting substance (GA_3) continued to rise throughout a 90 day period (Figure 4). Application of GA and N_6BA (a cytokinin) resulted in germination of non-stratified seeds when the seed coat was removed but had no effect on whole seeds. An extract from non-stratified seeds reduced growth of the radical of stratified seeds (Figure 5).

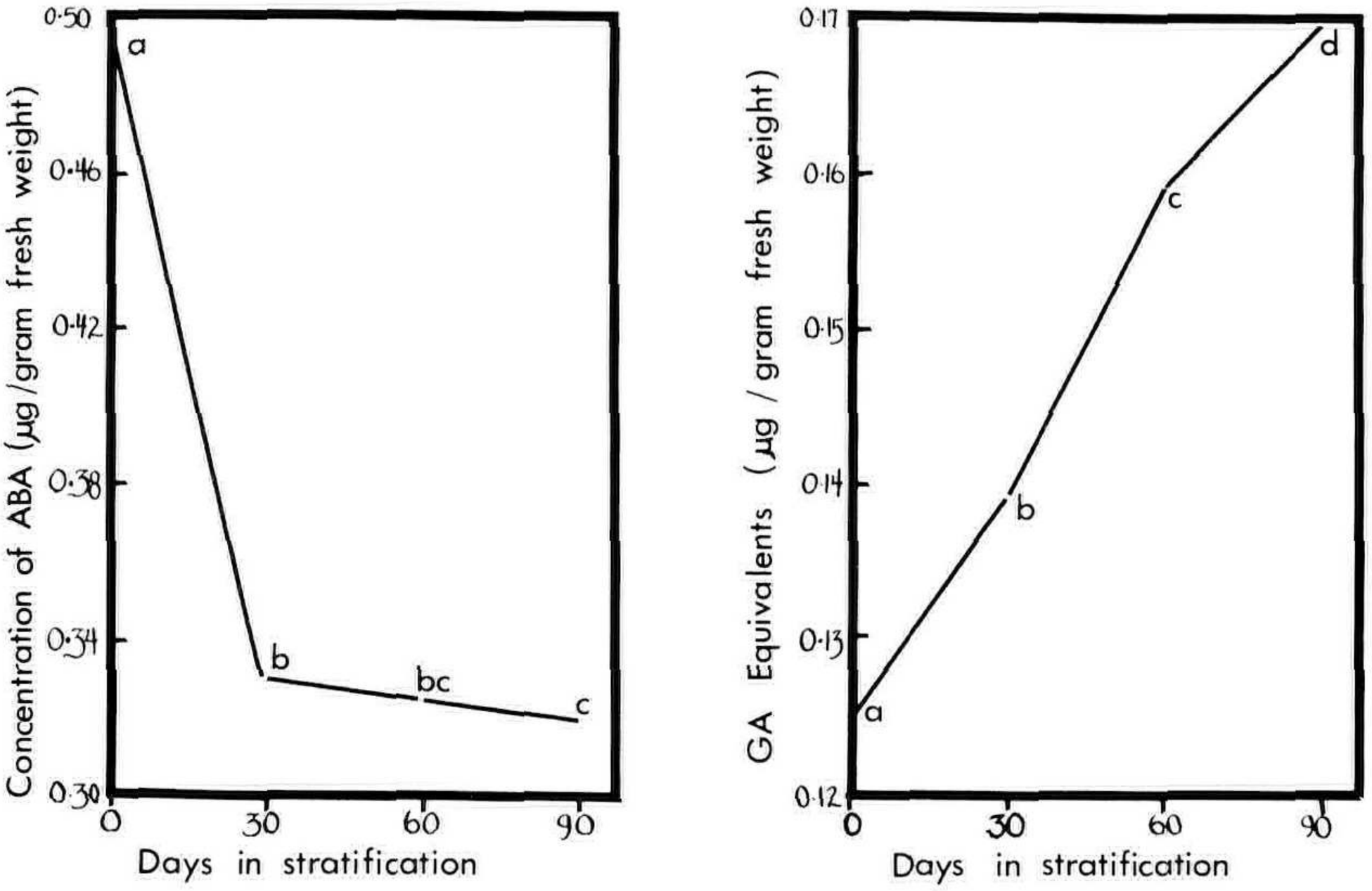

Figure 4. Concentration of inhibitor and promoter in plum seed after cold stratification of various time periods. From Lin and Boe (11).

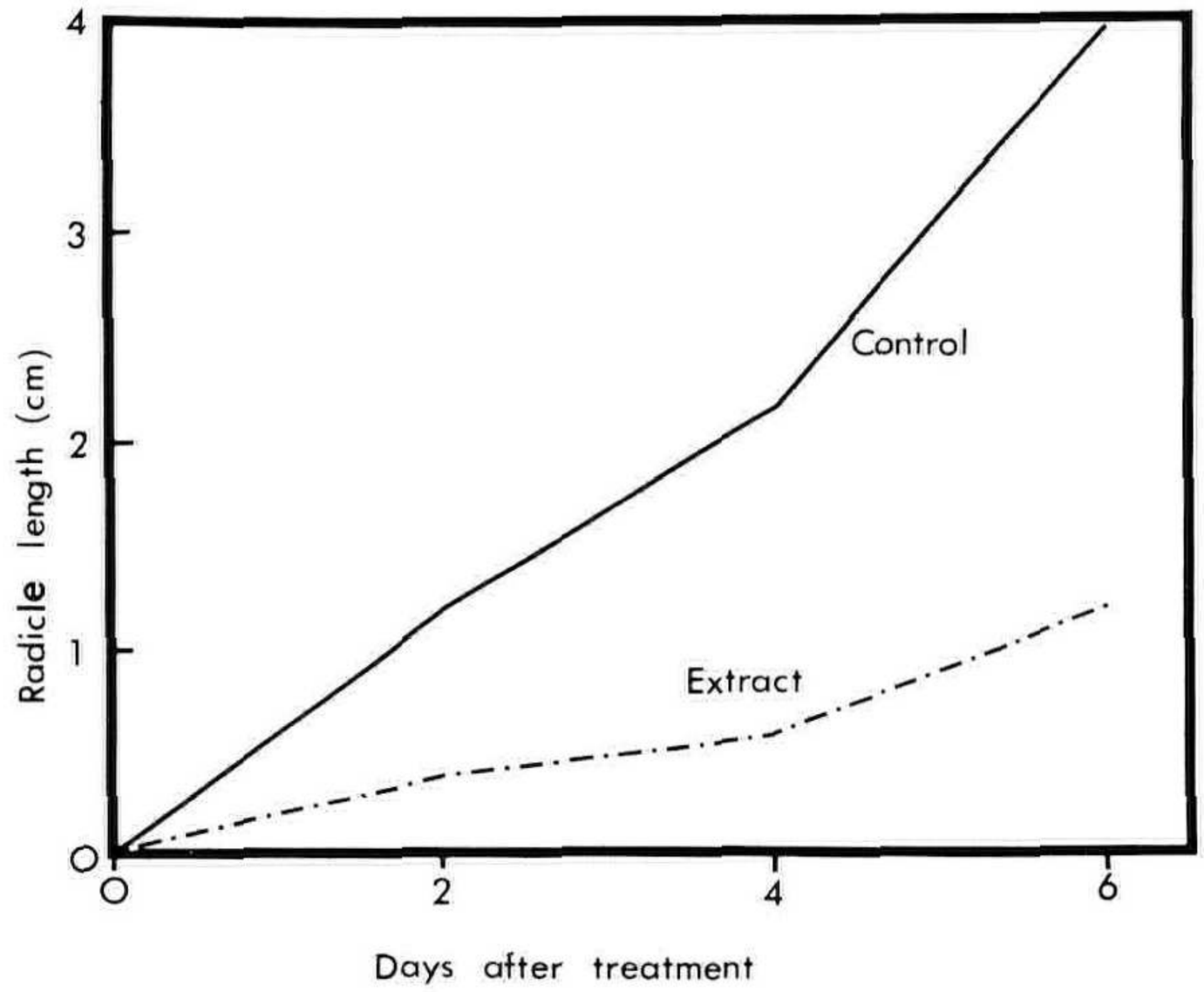

Figure 5. Effect of extract from non stratified plum seeds on growth of radical of germinating stratified seeds. From Lin and Boe (11).

Ross and Bradbeer (16) working with hazelnut (*Corylus*) found that the embryo in freshly harvested seed was not dormant but an inhibitor was present in the seed coat and pericarp. With dry storage, dormancy developed in the embryo. This dormancy could be attributed to the loss of promoter which occured during drying (Table 1).

Table 1. Levels of germination promoters (GA_3 equivalent) of *Corylus* seed collected fresh and after various dry storage and cold stratification treatments From Ross and Bradbeer (16)

	nmoles GA_3 Equivalent/seed	% germination at 20°C Naked Embryo	Intact Seed
Fresh seeds	87	77	16
28 days dry storage 2 days imbibed at 20°C	.02	12	3
28 days dry storage 28 days imbibed at 20°C	.01	12	3
28 days dry storage; 28 days stratification at 5°C	05	—	64
28 days dry storage; 28 days stratification at 5°C, 2 days at 20°C	94	—	64
28 days dry storage; 28 days stratification at 5°C; 7 days at 20°C	3 96	—	64

Concentration of promoter did not increase during cold storage, but increased rapidly (in 2 days) following removal from cold stratification. They found that a 28 day cold stratification or application of gibberellin could overcome the embryo dormancy that developed. Rudnicki (18) studying the inhibitor levels in apple seeds found that the ABA concentration dropped 20 and 40% respectively following 1 and 2 weeks of cold stratification. No ABA was found following 3 weeks of treatment. Exogenous applications of ABA inhibited germination of cold stratified seed, but greater concentrations of ABA were needed to inhibit germination as the length of stratification period increased (Table 2).

Table 2. Effect of applied ABA on germination of apple seed following cold stratification of various time intervals From data of Rudnicki (18).

ABA Applied ug/ml $^{-1}$	Days of Stratification 0	21	42	63	84
0.0	0	24.6	81.0	91.0	92.0
0.031	0	18.8	75.6	86.0	90.0
0.062	0	16.0	67.6	83.0	92.0
0.125	0	5.6	63.6	72.6	78.0
0.250	0	0.6	45.0	67.0	68.6
0.500	0	0	12.6	45.4	53.0
1.000	0	0	0.2	19.8	34.0
2.000	0	0	0	8.0	10.0
4.000	0	0	0	0	2.6

Webb, Wareing, and Van Staden (21, 24) found that 20, 40, and 50 days of cold stratification resulted in 15, 38 and 65% germination, respectively, of *Acer saccharum* seed. The greatest concentration of cytokinin was found after 20 days of stratification (Table 3), and the highest concentration of GA_3 was found after 40 days.

Table 3. Concentrations of cytokinin, gibberellins, and inhibitor in *Acer saccharum* seed following different time intervals of cold stratification From data of Webb, Van Staden and Wareing (24)

Stratification Days	ug/g Kinetin Equivalent		ng/g GA_3 Equivalent		ug/g ABA Equivalent	
	5°C	20°C	5°C	20°C	5°C	20°C
0	10	.10	2 0	2.0	826	826
20	5 92	1 19	23 0	21.0	—	—
40	1 18	70	37 3	12 3	—	—
50	99	57	4 0	27 6	018	373

In studies with *Acer pseudoplatanus*, Webb and Wareing (23) found that seeds with testa intact required cold stratification whereas bare embryos germinated immediately. The seed coats don't contain inhibitors but prevent leaching of inhibitors from the embryo. Kinetin at 1 mg/1 stimulated germination of intact seeds while GA_3 at concentrations of from 1 to 1000 mg/l had no effect. This again would fit the scheme proposed by Khan (10).

Thus, although naturally occurring plant hormones have been shown to play an important role in the regulation of the after ripening requirement of many seeds, it is quite apparent that an intricate balance of several hormones are involved in the control mechanism and that these conditions vary depending on the seed in question. Although there have been some positive results in use of the hormones, GA_3 and kinetin, as well as other chemicals such as potassium nitrate and thiourea to stimulate germination of some seeds, these chemicals have been only of limited effectiveness and will not give universal results as a substitute for the time-consuming and often cumbersome process of stratification. Looking on the bright side, there have been tremendous gains made in the knowledge of the role played by the plant hormones during the last 3 or 4 years. As our techniques improve and we learn more about the balances involved, I feel there is high hopes that in the not too distant future we will be able to apply a mixture of growth regulating chemicals to our troublesome seeds to achieve more consistent germination results.

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MODERATOR SHUGERT: Well done, Harold, our deep thanks. Tom Pinney, Jr. is next on the program speaking on "Seedbed Management".