NEW FRONTIERS IN PLANT PROPAGATION

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In considering the propagation of plants, sometimes it may be useful to back away from the details in which we often become trapped and get a broad, overall view of where we have been, where we are now, and then take a look ahead to see what is on the horizon that may lead to significant changes in the field of plant propagation.

There have been certain specific major developments in plant propagation history that, one by one, have greatly increased the kinds of plants that can be commercially propagated and have changed the economic picture of the nursery industry.

Prehistoric man may have discovered hardwood cutting propagation when he jabbed his spear cut from a living tree into the ground and found it starting to grow. Later, early man may have developed the rudiments of the technique we now call grafting. Presumably he could have thrown his spear at a wild beast and missed, with the spear jabbing into a tree. If, by chance, the combination of spear and tree was compatible, and if, by chance, too, cambial contact was made, the spear could have started to grow.

Propagation by leafy cuttings became practical when glass was discovered, permitting the propagator to maintain high humidity and keep his cuttings alive until they rooted.

A major step forward in plant propagation history occurred in the 1930's when plant physiologists discovered that auxins promoted initiation of adventitious roots on stem tissue.

In the 1940's and 1950's it was found that water mist sprays, by keeping the leaves of cuttings covered with a film of water, greatly reduced transpiration and prolonged their life. This led to "mist propagation", which almost revolutionized propagation by cuttings.

Turning and looking to the future, what do we see on the horizon? We find that some significant new developments are already with us, just waiting to be used in practical plant propagation.

One fast-moving area of research that many plant propagators are watching with great anticipation is variously known as tissue culture, *in vitro* culture, aseptic culture, etc. These procedures have been developing for a number of years and are now reaching the stage, especially for herbaceous plants, where the commercial nurseryman can make use of them for large scale propagation.

For example, Dr. Toshio Murashige at the University of California, Riverside campus, has been conducting a series of "short courses" for technicians employed by some of the larger California nurseries. He is teaching them the procedures involved in tissue culture, to bring the research developments into practical use in plant propagation. Some of these nurseries are establishing their own culture laboratories, and will be getting into commercial production using recently developed techniques. As an example of what can be done, consider the propagation of gerberas (*Gerbera jamesonii*). This plant is quite variable when reproduced by seed; and in the past, clonal propagation has been by the slow process of clump division. By using the appropriate tissue culture procedures, in 10 months, 1,000,000 gerbera plants, can be produced all exactly alike, starting with a single piece of tissue.

The various factors influencing regeneration in an herbaceous species must be studied before commercial use of tissue culture is possible. Such factors include: composition of the medium (which may vary at different growth stages), both for proliferation and organ regeneration; physical qualities of the medium — liquid, liquid with agitation, filter paper wicks, solid agar, etc.; light requirements — intensity (not too high), quality, and photoperiod; growing temperature; need for prior chilling temperatures to overcome dormancy; choice of plant part — stem section, shoot tip, axillary bud, etc. When the optimum levels for all these factors are determined, there is no reason why large scale, aseptic propagation cannot be used commercially, once the techniques are learned.

At the University of California at Davis, a group of biologists formerly associated with agricultural production departments has now formed a new Plant Growth Laboratory. Their intent is to become involved in the various areas of plant cell and tissue culture to bring the potential benefits of these techniques to agricultural production.

There are numerous exciting new possibilities for the use of these techniques in plant propagation. For example, *in vitro* excision and development of nucellar embryos into virus-free plants has already been accomplished. To avoid the juvenility accompanying such seedlings in citrus and to still retain the virus-free status of such seedlings, Murashige (13) has grafted, aseptically, clonal shoot tips onto germinated citrus embryos.

Other uses of aseptic culture which lie ahead for the plant breeder can subsequently involve the plant propagator, such as hybridization with somatic cells by enzymatic removal of cell walls and combination of protoplasts. So far, this has been done in only one instance, in which two tobacco species produced somatic hybrids with the same characteristics as sexual hybrids (3, 21).

However, efforts are underway in various laboratories to obtain hybrids, particularly of economically important crop plants, by such a method.

Another recent development in plant cell culture with considerable significance is the production of haploid plants from pollen grains. This is followed by diploidizing methods to form homozygous plants — a quick way to obtain "pure-lines" (24, 21). An important impetus to these techniques came from the studies of Nitsch (18), who worked out the nutritional requirements and the proper stage of pollen development, which is very critical. So far, plants in about 17 genera have been used for pollen culture and production of haploid plants.

Of course, some uses of tissue culture, such as shoot tip culture (mericloning) of orchids, have been with us for a number of years and are well-established as commercial practices (22). As far back as 1934, Tukey (23) aseptically cultured embryos from deciduous fruits.

The slow but steady increase in our knowledge of plant growth hormones is of immense interest to the plant propagator in all types of reproduction — by seeds, cuttings, and grafting. It seems that the plant propagator can find something useful in each new bit of information about growth hormones. He acquires a better understanding of what is taking place in the plant, and is better able to make use of synthetic growth regulators to exert control over plant reproduction.

Seed germination in woody perennial species is affected by numerous dormancy situations. It now appears that embryo dormancy, or "germination blocks", involves interactions of gibberellins, cytokinins, and inhibitors. This has been developed in a theory proposed by Khan (6) and supported by other workers (20, 25). Changing the balance among these three types of growth regulators can profoundly influence dormancy and germination (13), and could become a most useful tool for the plant propagator involved in germinating seeds of the woody perennial species.

What relationships will be found in the future between growth hormones and the rooting of cuttings? Are we about to find the mysterious rooting substance which, when added to auxin, is going to cause profuse production of adventitious roots? Probably what we are going to see is a gradual, bit by bit, development of knowledge of hormonal relationships that reveal why a little group of cells alongside a vascular bundle or just outside the cambium, differentiates into a root growing point. Today there is considerable active research in this field in many parts of the world, and a pattern of hormonal relationships is starting to appear. Fortunately, synthetic preparations of most of the known hormones are available, so we will be able to exert control by their use.

The pattern of interrelationships shown in Figure 1 (11) indicates the involvement of the several growth hormones in root initiation, according to evidence available today. However, there is still controversy as to the roles played by the several rooting "co-factors".

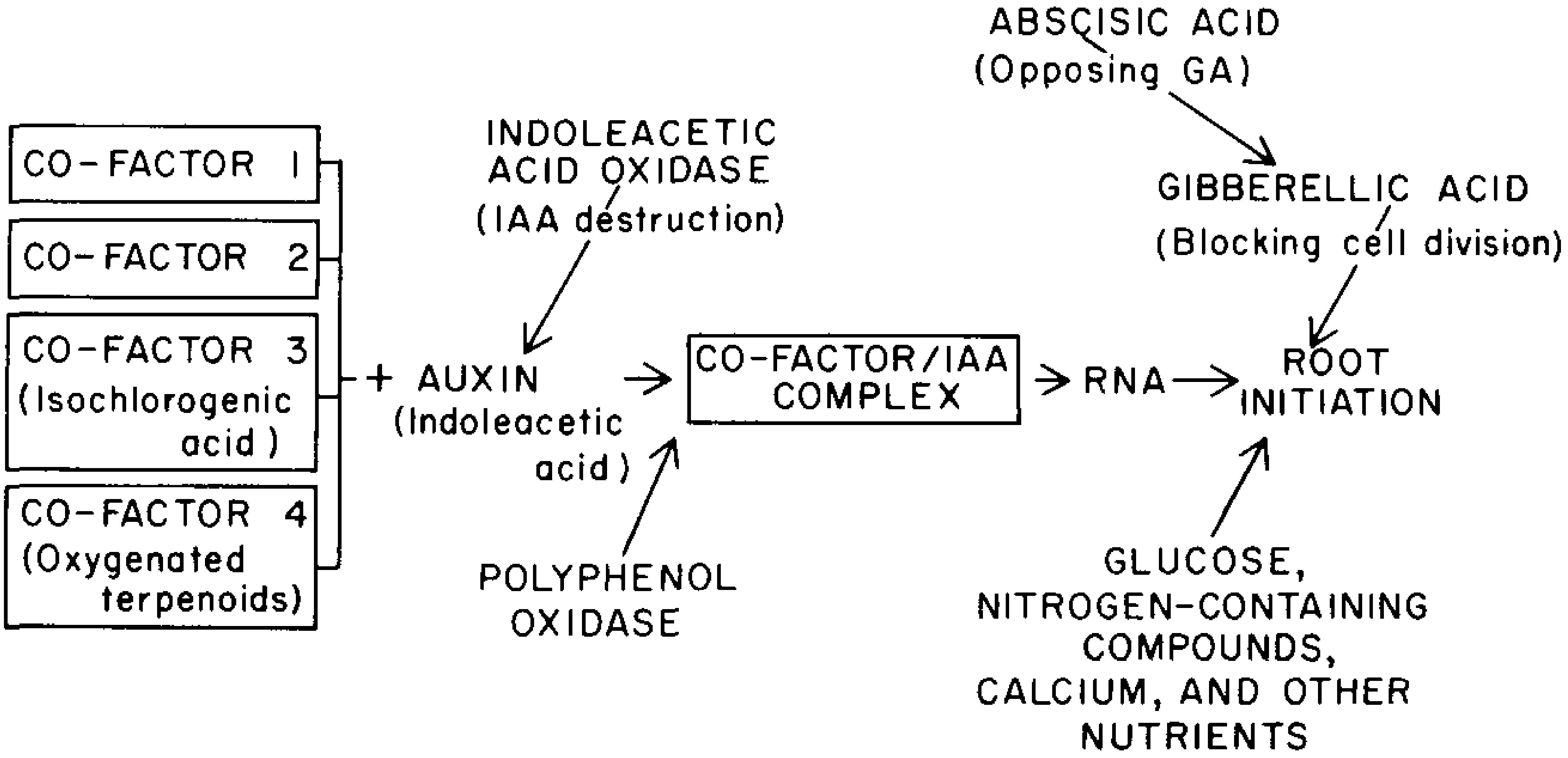

Figure 1. Hypothetical pathway of the interactions of various components leading to adventitious root initiation. (From Hartmann, Hudson T. and Dale E. Kester; *Plant Propagation: Principles and Practices*, 3rd ed. 1975. Prentice-Hall, Englewood Cliffs, New York).

Let us consider the involvement of ethylene in adventitious root initiation, and its relation to auxin. In 1933, Zimmerman (26) reported that auxin applications stimulated ethylene production, and suggested that ethylene could account for the activity of auxin in stimulating rooting. Are the ethylene-generating chemicals, such as ethephon, going to find widespread use in rooting cuttings? The present interest in ethylene as a growth hormone has included a new look at its uses in rooting cuttings and, as might be expected, the recent reports are not in close agreement. Krisnamoorthy (7), supporting Zimmerman's views, found that ethylene — released from ethephon — did stimulate rooting in mung bean cuttings. Mullins (14), on the other hand, also working with mung bean cuttings, found that ethylene did promote emergence of preformed root initials, but inhibited formation of additional initials. Kawase (5) noted an increase in ethylene levels, accompanied by an increase in rooting, in *Salix* cuttings as a result of centrifuging or just soaking in water. It has been well demonstrated, however, that ethylene will promote root development in cuttings and we now have easily applied ethylene-generating compounds so this is an area to watch in the future for possible important steps ahead in propagation by cuttings.

Is our increasing knowledge of hormone physiology helping us in the field of grafting? Many graft combinations give poor results chiefly because the plants involved are poor callus producers. We know from tissue culture work that certain combinations of auxins and gibberellin stimulate cambial activity. Here is a potentially fruitful area of research in which applications of certain combinations of growth regulators could have important beneficial influences in propagation by grafting and budding. From time to time, commercial preparations which contain some of the plant growth hormones, vitamins, amino acids, etc. become available for treating scions prior to grafting. Perhaps certain of these now under development will prove to be useful.

Some potentially significant studies illustrating how greatly increased growth of young seedlings can be attained by controlling the environment have been made at the USDA Plant Industry Station, Beltsville, Maryland. For example, increasing the natural daylength in the winter to a 16-hour photoperiod resulted in a dramatic increase in growth of paper birch [*Betula papyrifera*] seedlings. Further increases were obtained with additional light plus an increase in ambient CO_2 (10). Growth increases of 10 to 50 times were obtained with petunia seedlings by the use of higher temperature, light intensity, and CO_2 levels. Along with this increase in growth, seed to flowering occurs in 4 to 5 weeks (17, 19). Such environmental control of seedling growth may have great implications for the breeder of woody plants who would like to shorten the juvenile phase of his hybrid seedlings and hasten fruiting or flowering. Growth of crabapple seedlings was greatly stimulated by about a 10-fold increase in ambient CO_2, bringing plants from seed to flowering in 10 to 11 months (27). Although commercial production of woody nursery plants might be greatly accelerated by the use of such environmental manipulations, perhaps, the use of such techniques would have the most immediate benefit for propagators in the bedding plant industry.

It has been know for centuries that etiolation of stem tissues in conducive to production of adventitious roots, yet little is known of the physiological or biochemical causes of this effect. Etiolation should prove to be a fruitful field for intensive work to solve some of the mysteries of adventitious root initiation.

In a significant study by Krul (11) at the USDA laboratories, Beltsville, Maryland, there was evidence of photodecomposition of a root-promoting phenolic compound, 2, 4-dinitrophenol (an uncoupler of oxidative phosphorylation). This material was applied to the hypocotyls of bean plants, which were then covered with an opaque rubber tubing to block out light. This treatment strongly stimulated adventitious root production along the sides of the stem, especially when auxin was also applied. Such root formation did not occur without application of 2,4-dinitrophenol, or when the stems were covered with transparent tubing, permitting access of light to the tissues. Solutions of 2,4-dinitrophenol are stable to light; but with the addition of ascorbate plus chlorophyll (both naturally occurring plant materials), the 2,4-dinitrophenol decomposes to give 2-amino-4 nitrophenol, which was not effective in promoting root initiation when applied to bean hypocotyls in darkness. It could be concluded from studies such as this that withholding light from plant tissues (etiolation) may promote adventitious root formation by preventing photodecomposition of one or more naturally occurring rooting co-factors. Further studies of this type may provide information as to the nature of the elusive co-factors.

The influence of juvenility in adventitious root formation is a phenomenon which plant propagators have long been aware of, and have put to use whenever possible. However, we still do not know the underlying causes of juvenility in plants, much less why adventitious root initiation is so strongly related to plant age.

In looking for explanations for this phenomenon, the studies of juvenility in eucalyptus by Paton *et al.* (69) and Nicholls *et al.* (17) are most impressive. These Australian workers noted that leafy cuttings of all systematic groups of eucalyptus root easily but only if they are taken from young seedlings. The tropical *Eucalyptus deglupta* is exceptional in that cuttings from adult tissues will also root readily. (Eucalyptus species, in general, have marked juvenile-adult morphological characteristics.)

These workers extracted three closely related rooting inhibitors, found in high concentrations, from adult tissue of *E. grandis*. They determined by X-ray diffraction that one was a fused bicyclic compound of the ß tri-ketone type with a peroxide linkage. These compounds, when applied to stem cuttings of *E. deglupta* seedlings, which are normally easily rooted, strongly inhibited rooting. The three inhibitors were absent in the easily-rooted young seedling tissues of all eucalyptus species tested, as well as in the easily-rooted adult tissue of *E. deglupta*.

Perhaps we could conclude from this work that, in eucalyptus at least, the "juvenility effect" in relation to adventitious root production is associated with the synthesis of increasing amounts of rooting inhibitors as the plants age. Some plants, of course, may not synthesize such inhibitors in either young or old tissues so that tissue age is not an important factor in adventitious root production.

A relationship between levels of rooting inhibitors and ease of rooting in dahlia cultivars has been recently noted by Biran and Halevy (2). These inhibitors are produced in the roots and move upward into the stems, where they interfere with adventitious root production. If the identity of these rooting inhibitors could be established, applications of compounds to modify them chemically and nullify their action would be a significant step ahead in propagation by cuttings.

Some propagation practices have been used very effectively with some plants for many years, but these practices have not been developed to the level they could be with other plants, As an example, the use of heavily pruned hedge blocks as a source of cutting material for fruit tree rootstocks has long been a practice in England. But such practices are only now finding their way into cutting propagation of forest tree species.

In recent years there has been a considerable increase in interest in clonal propagation of forest tree species to preserve, for widespread use, distinctly superior single genotypes, either as producing trees themselves or for the establishment of seed orchards. Grafting is not the answer in all cases, due to the expense involved and to the incompatible relationships often found among individual forest trees of the same species, such as in Douglas fir. Some species, such as Monterey pine [*Pinus radiata*], are grown in great quantities in Australia and New Zealand as a lumber source. Thus, nursery trees, all originating from superior trees, are needed in great quantities.

Some significant studies with Monterey pine by Libby *et al.* (12), both in California and New Zealand revealed how maintaining heavily pruned stock plants can be of considerable benefit in cutting propagation of a coniferous plant. Monterey pine seedlings, by continuous hedging, developed into bush forms in contrast to large upright-growing trees. Cuttings then taken from the hedged plants rooted in high percentages, with good quality roots. Maintaining the trees in hedge form stopped the normal decline in rooting percentages with age. Perhaps such treatments assisted in maintaining a juvenile condition and, applying the rooting-inhibitor theory, prevented increases in inhibitor production. These hedging procedures may be worth testing with those woody plants species known to root easily when juvenile cutting material is used, but are difficult to root from cuttings taken from adult wood.

The use of aerated steam at a temperature of 140°F (60°C) can be a valuable procedure for treating soil (1) and seeds (16) prior to planting. The concept of using aerated steam to eliminate harmful pathogens was developed for practical use by Dr. Kenneth Baker of the University of California, Department of Plant Pathology, during a sabbatical leave in Australia in 1960. The Australian nurserymen, following his lead, generally went from no soil treatment at all directly into these most advanced concepts, so they are well ahead of nurserymen in many other parts of the world who practice high-temperature steam sterilization or chemical soil treatment. The problems inherent in high temperature (over 150°F) soil treatments, such as removal of beneficial organisms (which would hold an accidental pathogen contamination in check), liberation of manganese in toxic amounts, as well as the build up of ammonia and nitrites to harmful levels, are well known. Chemical soil treatments, although widely used, have their problems also. These are due chiefly to toxicity to humans from the use of such materials as methyl bromide and chloropicrin, as well as to effects of excess bromine in plants following the use of methyl bromide.

Development of simplified equipment for producing aerated steam will undoubtedly make its use more widespread. An ordinary centrifugal blower, with manually controlled steam introduced into the blower's air outlet seems to provide the basic requirements. Adjusting the steam pressure and a damper over the blower's inlet permits control of the steam-air mixture at the desired 140°F, ready for introduction into a plenum below the soil mass contained in an insulated chamber. Shutting off the steam, but permitting the air flower to continue, provides for rapid cooling and utilization of the treated soil.

The field of plant propagation is in the fortunate position of being able to benefit from research developments in several disciplines, e.g., plant physiology, biochemistry, genetics, botany, and plant pathology. The challenge confronting those in plant propagation is to keep abreast of newly developing research findings in these fields, and to pick out those which have a significant meaning in plant propagation and determine where they can best be utilized.

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