MYCORRHIZAE IN RELATION TO CONTAINER PLANT PRODUCTION

R.G. LINDERMAN

Ornamental Plants Research Laboratory
USDA-ARS, Oregon State University
Corvallis, Oregon 97331

The associations of beneficial fungi with most plant roots are called mycorrhizae and have been described by me and others in past meetings of the International Plant Propagators' Society (2,3,5,6,7,9,10,11,13), and in extensive literature. There is, however, considerable need to better understand the nature of these symbiotic relationships in order to exploit their benefits in commercial nursery production. The microbiological aspects of container production offer special opportunities for exploration as well as some special challenges. My purpose in this presentation is to briefly discuss our current thinking in relation to the establishment and performance of these fascinating fungi. In other words, how and when should we inoculate container plants, and what must we do to ensure their survival and maximize their chances to enhance growth and survival of their host plants.

Mycorrhizae: form and function. In general, mycorrhizae are of two types: **endomycorrhizae** and **ectomycorrhizae** (8). An understanding of their key characteristics is essential to an understanding of the problems encountered in their application to nursery plants. Endomycorrhizae are largely of the so-called "VA mycorriza type," occur naturally on a wide range of herbaceous and woody nursery crops, and are obligate symbionts (cannot be grown in artificial culture) and therefore inoculum must be produced in the soil on living plant roots. Ectomycorrhizae, on the other hand, can be grown in culture, much like mushroom spawn, and occur on a more limited host range including the families Pinaceae, Fagaceae, and Betulaceae (i.e., pines, oaks, and birches). Members of the Ericaceae associate with fungi that probably can be cultured but, to date, largely have not been.

My reason for stating these characteristics is that these groups of fungi are quite different and their biology and response to environmental factors and the methods of handling them in our mycorrhization efforts may be very different. We have become painfully aware of this fact after several years of experiments, many of which have failed. But these failures have in fact become stimulants to in-depth thinking and discussions and eventually productive experiments. I would like to highlight these research processes shared so completely

with me by several graduate students. I acknowledge significant contributions to this team effort by James Graham, Jennifer Parke, Brenda Biermann, and John Kough.

Mycorrhization: colonization and performance. Mycorrhization involves two main phases. Phase one is the inoculation of receptive host roots with viable inoculum with high potential to colonize the roots. The second phase follows the inoculation and colonization phase (actually the parasitic phase) and could be termed the extra-matrical phase. This phase occurs outside the root in the soil rhizosphere and beyond. This extra-matrical hyphal network will serve to bear new spores, but also becomes the feeder system through which water and nutrients are acquired from the soil and transported back to the host (Figure 1). Without this hyphal system the symbiosis may not become mutualistic. In other words, the fungus benefits by having a place to live and acquire carbohydrates, but it remains a parasite that doesn't pay its own way because it fails to help the host plant acquire needed water and mineral nutrients.

Figure 1. Extra-matrical hyphae (arrows) of *Glomus fasciculatus* extending from subterranean clover roots into the soil medium through which mineral nutrients are translocated from the soil to the root. (Photo courtesy of B. Biermann)

Until this last year, we assumed that if we observed the parasitic phase (colonization), as evidenced by morphological changes in the case of ectomycorrhizae, or by arbuscules or vesicules in the case of VA mycorrhizae, that the relationship was complete. We assumed that the extra-matrical hyphae had

been present but were removed during the straining procedure. But too often the plant seemed not to benefit by having "mycorrhizae." Why? The answer, we discovered, was that we frequently had complete root colonization, but apparently no extra-matrical hyphae to help the plant take up the phosphorus needed for growth.

The problem, then, was how to determine whether or not extra-matrical hyphae were present. The answer came to me one quiet lunch period as I perused a paper by Sutton and Sheppard (12) who had reported that VA mycorrhizal extra-matrical hyphae could aggregate sand-dune soil. Their focus was not on growth enhancement of the host plant, but their assay seemed to be just what we were looking for. The sticky extra-matrical hyphae actually bound sand grains together into aggregates. Thus, the more hyphae, the more sand grains were bound into aggregates. They shook off the non-aggregated sand grains through a screen that retained the aggregates which were then thoroughly washed off, dried and weighed The weight of sand grains was proportional to the extent of the extra-matrical hyphal network.

When I shared these thoughts with a former student, Dr. James Graham, a test system emerged that involved VA mycorrhizae on citrus and would test the hypothesis that the presence of extra-matrical hyphae was correlated with growth enhancement. It was known (J. Menge, unpublished results) that VA mycorrhizal fungus isolates from Florida for some reason failed to enhance growth of citrus grown in low P California soils, but native California isolates did enhance growth Dr. Graham ran the experiment in his California system and showed that Florida isolates colonized roots but failed to produce extensive extramatrical hyphae in California soils and correspondingly did not enhance growth, presumably due to the reduced hyphal network needed to acquire the P needed for growth (Figure 2) (4).

Thus we now have a new dimension to consider in our mycorrhiza research, but we also have the tool to measure it. These results serve to remind us that we cannot quantify mycorrhizae merely by counting morphological features, but must also attempt to determine whether the symbiosis is mutualistic, because there are soil factors which may prevent it from being so.

Soil factors that influence mycorrhizae. There are many factors in natural soil as well as environmental factors that undoubtedly influence the establishment and performance of mycorrhizae These are in addition to the host and fungus factors *per se*, although they may all be quite interdependent.

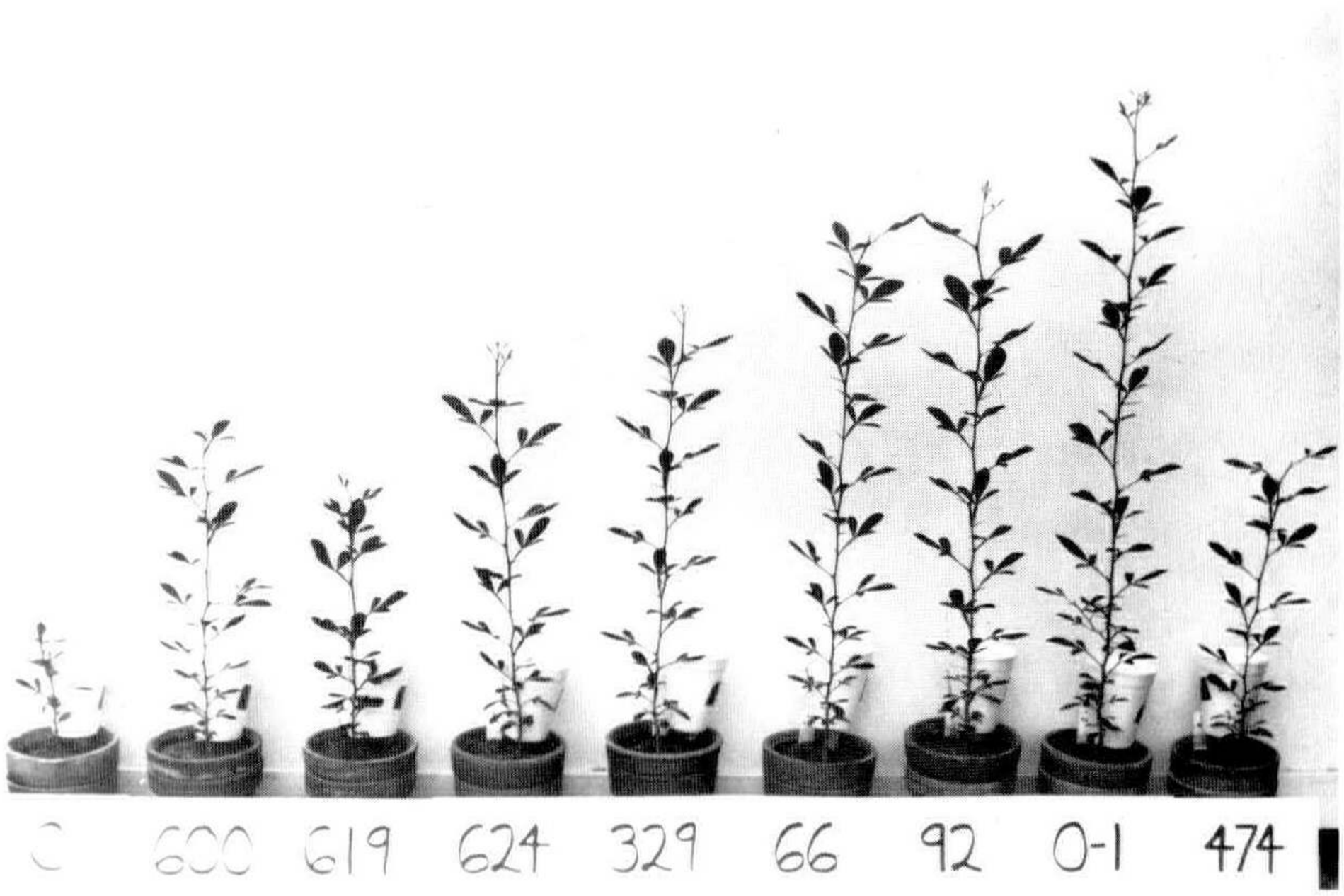

Figure 2. Growth response of Troyer citrange plants inoculated with different isolates of vesicular-arbuscular mycorrhizal *Glomus* spp. from Florida (isolates 600, 619, 624) and California (isolates 329, 66, 92, 0-1, 474) compared to the uninoculated control (C). (Photo courtesy of J.H. Graham).

For example, why did some isolates not form extra-matrical hyphae in one soil type while others did? We are currently testing a range of variables such as soil pH, organic matter content, pesticide content, nutrient levels, etc. Fortunately, in container nursery production, some of those variables can be controlled.

A series of experiments conducted by graduate student Brenda Biermann has focused on whether components of soilless container mixes influence the colonization and performance of mycorrhizae. She has found that VA mycorrhizae appear to be inhibited in soilless mixes containing peat, bark, vermiculite, and perlite. Further experiments suggest that these soilless mixes lack the P-fixing capacity that most soils have, so that too much P stays in solution and that inhibits establishment and performance of VA mycorrhizae. Adding some soil to the mix appears to nullify that inhibiting affect (1).

Helper Organisms: Another variable that we are especially interested in is the microbial complex in soil that is missing in container mixes or soils that have been pasteurized or sterilized by heat or gas. There is strong evidence to suggest that what we call "helper" organisms may be necessary in order for colonization to occur and for extra-matrical hyphae to form.

We are conducting tests to verify their importance and in the process isolate and identify them. Where would one find these organisms? We have hypothesized that if we can find a mycorrhizal association that is working, that we will also find organisms closely associated with the mycorrhiza and/or extra-matrical hyphae or rhizomorphs. If, by bioassay, we can find those that help mycorrhizae to form and function, then we should add them along with the fungi in the process of mycorrhization.

Thus we propose that there may be great benefit to "reconstituting" container mixes with organisms that we have chosen because of their ability to help plants grow. The most extreme need for reconstitution comes with tissue culture plants starting out in their "naked" state without the normal or natural complement of microbes in and on their roots. Our goal is to identify those organisms and learn how to add them to the system.

LITERATURE CITED

1 Biermann, B J and R G Linderman 1982 Effect of growth medium on establishment and performance of vesicular-arbuscular mycorrhizae on geranium and subterranean clover *J. Amer Soc Hort Sci.* (in press)

2 Camp, W H 1956 Micro-organisms in soils and their action on plants. *Proc Inter Plant Prop Soc* 6 107-121

3 Dangerfield, J A 1975 Mycorrhizae-plant relationships *Proc Inter. Plant Prop Soc* 25 105-111

4 Graham, J H , R G Linderman and J A Menge 1982 Development of external hyphae by different isolates of mycorrhizal *Glomus* spp in relation to root colonization and growth of Troyer citrange *New Phytologist* 90 (in press)

5 Holden, V L 1978 The use of mycorrhizae in the propagation of *Arctostaphylos uva-ursi* *Proc Inter. Plant Prop Soc* 28 132-133

6 Linderman, R G 1974 Potential use of mycorrhizal fungi to enhance growth and adaptability of ornamental plants *Proc Inter Plant Prop Soc* 24 86-91

7 Linderman, R G 1978 Mycorrhizae in relation to rooting cuttings *Proc Inter Plant Prop Soc* 28 128-132

8 Linderman, R G 1978 Mycorrhizae — indispensable aids to profitable plant production *Amer Nurseryman* 147(4) 17, 129-133, 147(5) 70, 72, 74, 78

9 Maronek, D M 1977 Mycorrhizae and plant growth *Proc Inter Plant Prop Soc* 27 382-389

10 Maronek, D M and J W Hendrix 1978 Mycorrhizal fungi in relation to some aspects of plant propagation *Proc Inter Plant Prop Soc* 28 506-515

11 Newcomb, D A 1975 Mycorrhiza effects following soil fumigation *Proc Inter Plant Prop Soc* 25 102-104

12 Sutton, J C and B R Sheppard 1976 Aggregation of sand-dune soil by endomycorrhizal fungi *Can J Botany* 54 326-333

13 Tinker, P B H 1973 Root-soil relationships *Proc Inter Plant Prop Soc.* 23 115-120

MODERATOR CHRISTIE· I am sure we have many questions for this panel. We are ready for the first one:

STEVE WALKUM: Dr. Tukey, in your slides you showed increased growth with Osmocote plus a foliar spray, compared to Osmocote alone. Were you using the optimal level of Osmocote, or was it suboptimal?

HAROLD TUKEY: The amount — I have to look it up in the paper exactly — but it was a good level, I don't remember what optimal would be, but it was a production level of Osmocote The amount of time I don't know, though the picture was taken one month after we had stopped the foliar nutrition. We ran the foliar nutrition for three weeks, then a month without any foliar nutrition. During that whole time the plant was receiving Osmocote So it would be seven weeks after the beginning of treatment that the picture was taken. The Osmocote, in that experiment, didn't give us any additional breaks.

STEVE WALKUM. How would you apply the spray on a commercial basis, how often would you have to foliar spray to supply the major nutrients?

HAROLD TUKEY: We didn't try to compare foliar versus root nutrition. These were just experiments to show the materials could get in. I don't know exactly what the timing would be in experimental trials, but the three weeks of foliar nutrition was enough to give the plants about 11 weeks of nutrients without anything additional, before the Osmocote in the root medium began to provide nutrients. Rhododendron growers talk about applying nutrient sprays to the foliage about every four or five days during the growing season, for bedding plants, about once a week, ground covers about once a week, or once every two weeks through the season. But I think the timing depends entirely upon the situation, how fast the plants are growing, the temperatures, and all the rest of many factors.

JUDY GARLOCK: For the small nursery, do you think that foliar application of nutrients, along with the regular soil application is practical and, if it is, how would a small nursery obtain the information that it would need to start a program of foliar feeding?

HAROLD TUKEY: Practicability — I am afraid that is your job, to find out whether it works in your system or whether it doesn't. I can tell you how to get the nutrients in, I can tell you the plants will grow. Whether you make money at it, that depends entirely on your situation There are many materials on the market that are perfectly good for foliar application,

and the directions are good. All the big fertilizer companies provide such materials, and if you want to get going, just pick up some of those. I could mention lots of them but I won't here. The way to start is just to try it. That means a small sprayer of some kind to see if it works for you. The first batch is going to cost you way too much; you will not be able to make it profitable but you will see if it works. That is how people go at it. There is also published information available. People are using proportioners — growers, particularly in California, Ohio, and Texas, have been using proportioners and putting on nutrients through overhead irrigation systems. They think it goes in through the roots, but it is also absorbed through the leaves. Ed Wood has some experience on adding nutrients by overhead sprinklers to forest tree seedlings. You can get some information from him. Ralph Shugert says keep a control treatment. Absolutely! Test it, don't go over everything with foliar sprays.

JOE DAVIS: Harry Lagerstedt, have you considered rooting the stock at the same time as the scion graft with your filberts?

HARRY LAGERSTEDT: I have tried to graft two cuttings together, as is done with grapes and citrus, and I have totally failed there. The graft union dried out in every case. But from that I deduce that the root system in the moist sawdust is providing some moisture to the graft union, which an unrooted cutting does not do.

VOICE: Bev Greenwell, is there a problem in bringing in root rot by using alder sawdust?

BEV GREENWELL: There hasn't been — no. The main problem we have had with sawdust is that it breaks down too fast, but there hasn't been a root rot problem. Once the sawdust is broken down it is a lot tighter, and then there may be more root rot, because the sawdust will be too wet.

RALPH SHUGERT: Bob Linderman — a question to you. I am confused on mycorrhizae. I have it in my mind that with a container medium, the higher the organic content, the more possibility of mycorrhizae. The slides you showed disproved that. For example, with higher peat percentages in the medium — I would think I would have more mycorrhizae, but your data didn't show that. Comment please?

ROBERT LINDERMAN: What plants are you talking about?

RALPH SHUGERT: A wide range of woody ornamentals, virtually all the conifers — *Juniperus*, *Thuja*, etc.

ROBERT LINDERMAN: I can't comment on the conifers,

which might have had ectomycorrhizae, because our experiments were related to VA mycorrhizae. As long as there is some soil, some clay to tie up part of the nutrients, then you tend not to have a nutrient inhibition of mycorrhizae.

The soilless mixes tend not to bind phosphorus. Phosphorus is known to inhibit both ecto and VA mycorrhizae. You have to bind part of what you are supplying or, if you water it often enough, you are going to send part of it through and not have an inhibition. But if the phosphorus remains there in solution, then it becomes inhibitory to mycorrhizae. That won't happen in all cases, but it could because the phosphorus is very available. It is not bound by most of the organic amendments that you are talking about. Peat moss or bark tend not to bind the fertilizer, they keep it in solution, therefore, if you have a lot of rain it is going to wash through and you are going to have to fertilize more often. But if you keep it in solution, it is going to inhibit mycorrhizae. That seems to be the effect. If you add some soil or clay, which have a high fixing capacity, then the phosphorus is chemically bound to those particles, and you tend to have less phosphorus in solution, therefore less inhibition. Does everybody understand that? I had to go over this very fast, but there is a phosphorus inhibition of most mycorrhizae, so that if you have phosphorus in solution and available, it will tend to inhibit the mycorrhizae. You can have more phosphorus available in soilless mixes than in a mix that has soil added to it. Even pasteurized or sterilized soil, or some other clay particles that have a greater fixing capacity, will nullify that effect.

HAROLD TUKEY: I was too quick to make fun of my friend from Washington. You might not expect to necessarily find an advantage with foliar nutrition, if you are doing a good job with root nutrition. Foliar application doesn't offer anything magic. If you get different materials into the leaves that are not going in through the roots, then you will get an effect. If you are doing a good job through root nutrition, you can put all you want onto the foliage, but you probably are not going to get much change.

JIM SAHLSTROM: I would like to ask Dr. Ryan a question. We use Devrinol about three times a year in our nursery through the sprinkler system. Once in a while I have a feeling we may be getting it on our bedding plants. How much can we put on our bedding plants without damage to them? And also perennials, I would like to use it on that.

GEORGE RYAN: I can't really answer that. I have had no experience with it on bedding plants or perennials. There is some work being done on that at various places across the

country, but I can't answer the question. I would only say to contact the Stauffer representative and see what they say about it.

GEORGE MATSON: What is the optimal time or method for inoculating plants with mycorrhizae — in the seed flats, or in containers, or do you just mix it in your soil?

ROBERT LINDERMAN: My general attitude has been to inoculate at the earliest possible time. That is why we have looked at tissue culture — or at time of seeding. There is a problem with longevity of inoculum because of the form one has to use. So, in Georgia, Don Marks can inoculate ectomycorrhizae pine trees, he can inoculate them at time of seeding, and the inoculum stays alive and well until there is a root to receive it. In the Pacific Northwest if we use the same technique, inoculate at the time of seeding, the inoculum is worn out, or the inoculum potential is way down by the time there is a root to be infected. So, that is really the rule of thumb. If you can get the inoculum together with a receptive root in a minimal amount of time, that is the best. So, if you are sticking a cutting, and you don't have roots yet, there is a chance that the inoculum wouldn't be any good by the time you did get roots two months later. So in that case, I would rather be adding the inoculum after you already have a root ready to be infected. If you are talking about tissue culture we would like to have the mycorrhizae right there as soon as roots form; that is why, in our experiments, we are taking cuttings right out of phase 2 tissue culture, rooting them directly in a vermiculite medium, and, as soon as they root, get the inoculum there — that is the best situation. There are logistics problems for tissue culture people to get those two things together at the right time. That is the reason that we are doing research on it.

STUART FRASER: Question for Dr. Tukey. Is there any relation on the effectiveness of foliar feeding to leaf surface, any work being done on conifers, or one particular interest of mine — *Calluna*?

HAROLD TUKEY: I can't speak specifically — conifers do show foliar absorption, but there is some injury. We have noticed, quite surprisingly, that some of the junipers are injured relatively easily by foliar nutrition — at rates that the deciduous materials can handle very well. You always think of good old junipers being rather tough, but as far as the foliage they apparently are not. They absorb nutrients nicely, and the injury that appears is peripheral but new growth develops easily. But I don't know the reasons for the injury. It isn't just the thickness of the cuticle, because the cuticle in some coni-

fers is about the same as in other plants, so there is no relationship there. It has to do with the cuticle make-up and we haven't looked at that. I am sure that there is a relationship, but I don't know what it is.

VOICE: Question for Bev Greenwell. Regarding container-grown azaleas in the Pacific Northwest, do you think 10 lbs is the optimum rate of 18-6-12 Osmocote?

BEV GREENWELL: We had good results at 10 pounds and even as high as 12 pounds, but that is with very careful watering. One should monitor their own salt content if they are going to those rates. What you are doing is hovering at the very peak between optimum growth and toxicity. And if you sneak over into the toxicity range, you lose any advantage of going to the high rate.

VOICE: How about just 8 pounds to be safe?

BEV GREENWELL: OK — you are going to be safe, but then you are getting down to where you have fairly good looking plant material, growing at a reasonable rate, but you are actually getting hidden hunger symptoms — where you aren't getting optimum growth, but they look OK. You are safe but it might take you an extra year to grow the plant.

WESTERN REGION 1981 AWARD RECIPIENT*

PRESENTED BY BRUCE USREY

The individual we honor today for the Western Region Award of Merit has achievements so extensive that it is hard to choose where to start. His achievements cover more than a quarter of a century. One marvels that an individual could be involved in so many projects to benefit his industry and fellow man.

He has always been alert to new processes and procedures. Always among the first to experiment with new equipment and new supplies in an effort to produce a better plant. The propagation of plants by faster, more efficient methods is always a challenge. He has found a special challenge in tissue culture and is commercially producing many hundreds of thousands of plants by this method, including conifers, berry vines, apple trees, *Kalmia*, a long list of *Rhododendron* cultivars, and many other plants.

---

* Presented at the Western Region 1981 banquet.