

## AUGUST 1987

Editorial Committee: BOB HEATH  
THERESA HEATH  
ARNOLD STARK  
LILLIAN STARK

If you enjoy this newsletter, if you benefit from membership in OUR RFCI, and if you want all this to continue, then you must appreciate how important your support of our tree sale is. WE NEED YOU: to post the enclosed fliers in public places, to come to the September planning meeting, and to share the work of set up, sales, and cleanup. REMEMBER: WITHOUT YOU THERE IS NO RFCI.

## TISSUE CULTURE &amp; THE ORLANDO SEEDLESS GRAPE by Dr. D. J. Gray

Dr. Gray opened his talk with an explanation of why we cannot grow table grapes in Florida like those we find in the supermarkets grown in other parts of the country. In Florida when we plant traditional viniferous cultivars of grapes, they always succumb to a disease known as Pierce's Disease, which is a leaf hopper-borne bacterial disease. This is really related to our tropical climate so that the reason we can't grow these grapes in Florida is due to their lack of adaptation to our climate. To get around this they are trying to breed commercially acceptable grapes in Florida so they will have Pierce's Disease resistance and other types of disease resistance.

The way they have done this is by hybridizing viniferous and labrusca cultivars with native Florida bunch grapes. As we all know there are many kinds of Florida bunch grapes which grow wild in Florida. What very few people know is that the University of Florida has been in grape breeding for 43 years and at this time has released approximately 10 cultivars of grapes that range from jelly to wine grapes and all the way to the latest seedless variety called Orlando Seedless. The most recent release is a wine grape called Blanc DuBois which is producing a premium white wine that has been accepted well by the French and Californians.

Dr. Gray was hired by IFAS in Leesburg about 3 years ago as a bio-technologist which means that he is not involved in gene splicing and such as that but rather in tissue culture and hybridizing, with which we are all somewhat familiar.

The entire thrust of their program is to accelerate the development of grapes in Florida. There are several areas which can be explored with tissue culture and with a little bit of luck will greatly accelerate the development of cultivars of grapes. They have 3 different programs under development. One is the micro propagation of grapes which is a method of rapid propagation to enable the release of new cultivars very rapidly. The second is the accelerated development of seedless grape cultivars by embryo rescue. The third is in-vitro selection and propagation of grapes by somatic embryogenesis.

At this point Dr. Gray began his slide presentation to explain the above three methods of grape propagation. Previously, when grapes were released from Leesburg, the center had 10 to 20 vines growing at the center to provide cuttings to the interested nurseries. Vines are sold to the nurseries at a very nominal price. At that point, it takes two to three years for sufficient vines to be available to meet normal demand of the public. A good example is the Orlando Seedless which was released two years ago and today there are still nowhere near enough grafted plants for the demand. One way to get around this is to use tissue culture in the initial propagation phase which theoretically could produce a million plants in 6 months. This, of course, is impossible because of the manpower restraints in handling a million plants. With the tissue culture technique, we start with potted grape plants in the greenhouse because it is cleaner than operating in the field.

The first slide showed the potted grape plants growing in the greenhouse and they are pruned repeatedly to produce many shoots because it is the end of the shoot, the meristem, which is used in tissue culture and a maximum number of shoots provide a maximum number of cultivar pieces. The ends of the stems are taken into the laboratory where they are cut down to size. The pieces at this point are surface sterilized in a solution of Clorox which is standard tissue culture procedure. They are then put under a microscope and dissected micro-surgically in a sterile environment as was indicated by the next slide. The slide showed a technician sitting at a laminar flow hood peering through a microscope and holding tweezers in one hand and a scalpel in the other. The small tip is cut out and placed on an agar medium. The pieces at this point are 1 to 3 millimeters, very tiny. The medium is a standard tissue culture medium which contains sucrose as an energy source and all the salts and nutrients needed for plant growth, plus a growth regulator which will cause the tissues to produce a multiple type of growth with many shoots.

The next slide showed what a culture looked like after about a month. Little plants growing rapidly in several directions. The slide was of Orlando Seedless, which branches well. Muscadines tend to produce less branching. At this stage in order to increase production, the growing meristem is placed in a new medium where, by careful trimming, more shoots can be propagated. This enables them to produce shoots in vast quantities, as many as they are able to physically handle. In order to bring the shoots out, or in other words, produce plants from the shoots, the shoots are cut off and placed on another medium which contains a different growth regulator which helps in the production of roots.

The next slide showed the little plants producing roots in the medium and the next slide was a chart of the different grape cultivars that they have been growing in Florida. All in all, they've done about 35 different grape crosses, or grape species cultivars but the charts only showed those that have been grown under standard commercial conditions in Florida. The chart showed the Black Spanish which is a grape that is being used for wine, Carlos and Dixie which are two muscadine cultivars. Daytona is a reddish bunch grape, Suwannee is a good brown grape, and Orlando is the only seedless table grape that has been released. The chart shows the number of shoots that are produced on an average by each different type of grape.

The next area they have been working on is accelerating seedless bunch grape cultivars, with tissue culture being a very nice application in this area. The problem with breeding seedless grapes is that when you cross a seedless with a seedless, you get no seeds. There is no way, for instance, to cross Orlando Seedless with a Thompson or Flame. So the way that conventional seedless grape breeders get around this problem is to use a seeded female with a seedless pollen, get pollination, produce the grapes, plant the seeds and select those grapes with good qualities and some reduction in seededness. Take that grape and cross it with a different seedless pollen and so on until, what is eventually hoped for, we get a seed which will produce a seedless grape. This may seem a little far-fetched but it works and that's the way the Orlando Seedless was produced. One problem with this method is that it takes from 8 to 10 years to accomplish and also you can't make the real crosses you want to make. You can't cross Orlando with Thompson or Flame. So what does happen when you cross one seedless with another seedless? Pollination does occur, fertilization also occurs. A zygote is formed and an embryo starts to develop. But the genetic block to seed development stops the development of the seed and at some later stage the ovule aborts, which is what leads to seedlessness in the seedless grape. If you cut open a Thompson seedless and look very carefully at the stem end, you will see little white structures which are called ghosts and which are the aborted ovules of the seed. The application of tissue culture requires that we go into the grape before the embryo aborts and rescue that material while it is still viable and culture it to cause development to occur. This is called embryo rescue or ovule culture and the next slide showed this method being used. This is probably the most successful part of their program at Leesburg because they have about 500 of these grapes in the ground right now.

The next slide showed a bunch of grape buds before the flowers have opened, which is the stage at which pollination is conducted. In order to be certain that you are making the cross you want, you have to take the flowers before they are opened and remove the calyx and the male anthers, leaving only the female parts. It is a tedious process and when they first began, it took about  $1\frac{1}{2}$  hours to do one bunch. Now they have breeders who can do a bunch in about 20 minutes. So the breeders are doing all of this kind of work. Periodically, the flowers are treated with pollen and then bagged to protect the bunch from other wayward pollen and then they wait for the grapes to develop. They have found that the ovules do not abort until just before the grape begins to swell and ripen. This is the time, before the ovule aborts, when the grapes are removed to the laboratory. They are sterilized in Clorox, rinsed with water and then cut open.

The next slide showed a young grape cut open. We could see the ovule which was quite large in proportion to the berry because the grape was only 1/3 to 1/2 its mature size and the ovule was at full size. The ovules are removed and placed on a medium which is a completely different medium from the one used in previous tissue culture. It contains charcoal, gibberellic acid and some other things. They are then placed under light and the ovule, which would not normally do so, turns green and becomes photo-synthetic. The ovule has the shape of a seed but it is not becoming a seed, it is quite different. They are incubated for about 3 months like this during which time they develop considerable calloused areas which was apparent in the next slide.

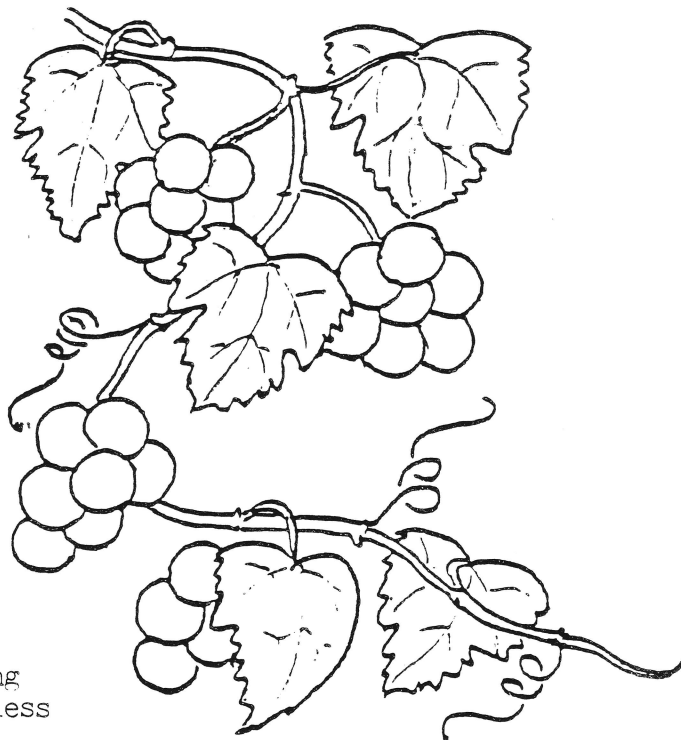
At this point the ovules are carefully opened and inside they frequently find a well developed embryo. The interior of the seed looks quite normal. The embryos frequently look quite different, some with fully developed endosperms, some with aborted endosperms, and some not developing at all in an empty cavity. What is interesting is that they can make all the embryos develop into plants. At this point, the embryos are all removed and placed on a different medium that has a growth regulator that causes the embryos to develop, which was indicated on the next slide. This is very interesting because they can see what occurs inside a seed during development which otherwise, of course, one cannot see.

The next slide showed further development of the embryos with cotyledon developed and the seeds sprouting and some with roots and two leaves following the cotyledon. At this point, they are moved into the culture boxes and allowed to develop further.

The next slide showed some of last year's crosses growing in the soil. So far, since they have begun this program, only two vines have fruited out of many hundreds they are developing and of these two fruiting vines, one is seedless which is 50% success.

So far they have made 3500 ovule rescues of seedless grape crosses and from that they have produced approximately 200 plants. This is a lot of work but theoretically their percentage of seedlessness will jump from about 17% to about 70%.

One primary objective beyond the production of a Florida seedless bunch grape is to put seedlessness into the muscadine. Last year they crossed many muscadines with seedless pollen and got a few grapes out. The problem with this is that muscadines have two more chromosomes than bunch grapes so when you make the cross you have one too many or one too few no matter how you look at it and something has to happen, either that chromosome has to get lost, which moves it down to the bunch grape level, or the chromosome has to duplicate itself, which moves it up to the muscadine level. They have found that using the muscadine as the female is a very difficult cross while using the bunch grape as the female with the muscadine male pollen is much simpler. They are hoping to get an intermediate plant that is a muscadine type and seedless, and cross it with muscadine to move it back into the muscadine group and end up with some high quality seedless muscadines. The Muscadine people in Mississippi who have a muscadine processing plant have expressed much interest in a seedless muscadine.





The third procedure which Dr. Gray explained is the latest developed and one that hasn't begun to pay off yet. It is a portion of tissue culture called somatic embryogenesis. Plants are the only organisms that produce embryos from a single parent's tissues, from one plant's cell, which are called somatic cells. So an embryo from a somatic cell is called a somatic embryo. The uses of this process are (1) to clone plants with seeds so that we have the convenience of the embryo within the seed and (2) to use these with genetic engineering tools. The slide showed one of the applications called variant cell line selection. We start with a population of totipotent cells which are cells that can produce a complete organism. If we regenerate the single cells of plants with different genotypes, we can use a screen or selection agent to remove all the undesirable cells. For example, we could treat these cells with Pierce's Disease toxin and kill off everything except the very rare mutant that is resistant to Pierce's Disease. Then proliferate these cells and grow them into plants which would, of course, have a resistance to Pierce's Disease. The key here is that instead of breeding two plants together and trying to select the resistance that we want, we merely take one parent, render it resistant and grow that exact same grape out except that now it is resistant to Pierce's Disease. So the idea here is to bring desirable table grapes such as Flame Seedless into Florida as a pure cultivar except with the resistance to Pierce's Disease. People have been working on this for 43 years and we haven't come close to developing Pierce's resistant grapes with conventional breeding. Once they get all this in place, it should only take a few months for the actual selection. However, they do not have all this set up at this point.

They start with flower parts, anthers, young ovaries, which was shown as a culture in the next slide. It didn't look like much but in the culture were masses of callouses growing on the flower parts and occasionally somatic embryos, which are white spots we could see in the slide. These can be carefully manipulated by culture media selection and in the culture we find classic stages of embryony all developing from single cells. We could actually see some embryos with two cotyledons which will, of course, develop into plants. The idea here is that at an earlier stage they can add the screening agent and they will be able to select out of vast populations of individual cells a single disease resistant cell and then grow the embryo out, forming plants which can be taken out to the greenhouse. They are very enthused about this approach and working diligently to perfect it.

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#### FROM THE PRESIDENT

Some sources of information on rare and unusual fruits and vegetables are available in your libraries and bookstores. Here is a list of some I have read lately:

The July issue of "Scientific American" has an article on wind pollination.

The May issue of "National Geographic" has an article on kiwi fruit.

The June issue of "Smithsonian" has an article on preventing foreign plant pests from invading our borders. Those of you who travel abroad and bring back plant material should look this one up.

The July issue of "Natural History" has an article on a viroid coconut disease in the Phillippines.

The July-August issue of "Plain Truth" is devoted to agriculture, and has information on "Little-Known Food Plants. Shrubs and Trees with Great Potential".

"Sunset" has published "Fresh Produce A to Z", with pictures, descriptions and recipes for many of the newly available fruits and vegetables.

Harper and Rowe has published "Uncommon Fruits and Vegetables: A Common Sense Guide", by Elizabeth Schneider.

There will not be a test on the above but try to read them anyway!

JULY PLANT RAFFLE

Plant Name	Donor	Winner
Rollenia	RFCI	Maya Byvoet
Yellow Guava	H. Hill	J. Murrie
Chinese Jujube	B. Heath	Al Hendry
Stover Grape	RFCI	Herb Hill
Rootbeer Plant	Maya Byvoet	Nels Gullerud
Ground Cherry	Nels Gullerud	Bob Heath
Ground Cherry	Nels Gullerud	Stark
Papaya	Nels Gullerud	Maya Byvoet
Monstera Deliciosa Fruit	Maya Byvoet	Walter Vines

RECIPE OF THE MONTH: GERMAN APPLE CAKE (from a 1907 cookbook, "Fruit Recipes")

Mix 2 cups flour with 1/2 tsp. salt, 1 Tbs. sugar, and 2 tsp. baking powder. Cut in 2 Tbs. butter. Stir in 1 beaten egg and 3/4 to 1 cup milk for a thick batter. Spread in a buttered pan, 1" thick. Over top arrange pared, sliced apples in rows, sharp edges pressed into the dough. Sprinkle with 3/4 cup sugar mixed with cinnamon, and butter. Bake at 400° for 40 minutes.

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## JULY HOSPITALITY TABLE

Bea Seekins - German Apple Cake  
 Pearl Nelson - Zucchini Bread  
 Lillian Stark - Banana Nut Bread  
 Nels Gullerud - Fresh Ground Cherries  
 Felicia Mendez - Beverage

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TAMPA BAY CHAPTER  
 RARE FRUIT COUNCIL INTERNATIONAL  
 P O BOX 260363  
 TAMPA FL 33685



P. JUDSON NEWCOMBE  
 314 DEER PARK AVE.  
 TEMPLE TERRACE, FL 33617