This report serves as an update on the progress that has been made in my laboratory in recent months on the project "Horticultural Performance of Ethylene Insensitive Petunias", funded for the 1999 research year by The FNGA. As a result of experiments conducted with these funds, we have made progress on all goals set forth in all initial proposals.

The overall objectives for the project ***Horticultural Performance of Ethylene Insensitive Petunias*** have been as follows:

1. **Genetically transform inbred lines of petunia with the dominant mutant *ETR1-1* gene from *Arabidopsis thaliana*.**
2. **Breed the mutant *ETR1-1* gene to homozygosity using traditional breeding methods.**
3. **Evaluate horticultural performance of ethylene insensitive (*ETR1-1*) progeny in relation to commercial success.**
4. **Devise methods to engineer tissue specific ethylene insensitivity.**

**RESULTS & DISCUSSION**

**Objective 1 – Genetic transformation of inbred petunia lines with the dominant mutant *ETR1-1* gene *from Arabidopsis thaliana*.**

Transformation of petunia using *Agrobacterium tumefaciens* is now routine in our laboratory and we are now able to efficiently transform petunia with any genetic construct of interest.

**Objective 2 - Breed the mutant gene to homozygosity using traditional breeding methods.**

We have now bred all of our important ethylene insensitive *CaMV35S-ETR1-1* lines to homozygosity, and have bulked up quantities of seeds with the purpose of making them available to any floriculture researcher interested in using them for research purposes.

**Objective 3 - Evaluate horticultural performance of ethylene insensitive (*ETR1-1*) progeny in relation to commercial success.**

In combination with Objectives 1 and 2 described above, we have acquired much data on the horticultural performance of ethylene insensitive (*ETR1-1*) plants. Two graduate students have completed their MS thesis working on these projects.

**Pertinent research questions answered in these graduate theses:**

- *Do the ETR1-1 plants flower in an acceptable amount of time?*
- *Do the ETR1-1 plants have the same floral display as normal plants?*
- *Do ETR1-1 flowers last longer after ethylene treatment or pollination?*

- *Do ETR1-1 flowers last longer in the landscape?*
- *Do ETR1-1 plants reproduce normally and what is their rate of fruit set and seed yield?*
- *What is the rate of fruit ripening in ETR1-1 plants and what is the appropriate procedure in making crosses for maximum seed production?*
- *Do ETR1-1 plants produce viable seeds?*
- *Do cuttings taken from ETR1-1 plants form normal adventitious roots?*
- *Does ethylene sensitivity affect pollen viability and pollen tube growth?*
- *Does ethylene sensitivity affect seed production and seed germination?*

**Do the *ETR1-1* plants flower in an acceptable amount of time?**

Yes. We have seen no difference between *ETR1-1* and wild type plants for the time it takes for a seed to germinate and grow until the first flower reaches anthesis.

**Do the *ETR1-1* plants have the same floral display as normal plants?**

Yes. We have seen no difference between *ETR1-1* and wild type plants for the number of flowers per plant.

**Do *ETR1-1* flowers last longer after ethylene treatment or pollination?**

Yes. *ETR1-1* flowers always last longer when placed in a jar and treated with ethylene. For pollination induced corolla senescence, it depends on which line is being examined and which environment it grows in. Some *ETR1-1* lines last longer than others. In more stressful environments (high light, temperature and humidity), pollination-induced flower senescence is delayed in *ETR1-1* plants, but not to the same degree that it is under optimal conditions (high light, lower temperature and humidity).

**Do *ETR1-1* flowers last longer in the landscape?**

No field testing results have been obtained to date, but we know from greenhouse experiments that environment affects the length of flower longevity we see. In more stressful environments (high light, temperature and humidity), natural corolla senescence is delayed in ETR1 plants, but not to the same degree that it is under optimal conditions (high light, lower temperature and humidity).

**Do *ETR1-1* plants reproduce normally and what is their rate of fruit set and seed yield?**

Fruit set is decreased under stressful environments – under normal environmental conditions fruit set in *ETR1-1* plants is normal.

**What is the rate of fruit ripening in *ETR1-1* plants and what is the appropriate procedure in making crosses for maximum seed production?**

We have observed a delayed fruit ripening phenotype similar to that of Never Ripe tomato, which in turn increases seed production time by approximately 20%. We have also observed that seed germination rate is decreased on fresh seeds harvested from ethylene insensitive maternal plants. Currently we are using homozygous inbred lines and wild type plants to make all conventional breeding crosses (selfs, sibs, reciprocals). This will allow us to determine if problems with seed set and seed germination can be overcome by using *ETR1-1* plants as pollen parents and wild type plants as maternal parents in crosses leading to production of F1 hybrid seeds. With the dominant nature of the mutation, it is apparent that breeders would need to use the *ETR1-1* plants as pollen parents in making F1 hybrid seeds.

**Do *ETR1-1* plants produce viable seeds?**

Yes, seeds produced from *ETR1-1* plants are viable, but need to be treated with $GA_3$ to germinate normally, especially when harvested fresh and sewn shortly thereafter. We have observed that *ETR1-1* seeds germinate better when allowed to "mature" after harvest. We are currently working to produce enough seeds to quantify this effect, and we are also working on seedling vigor experiments with *ETR1-1* and wild type plants. All of this work is being conducted with seeds produced from crosses made in the previously described work on the appropriate ways to breed these plants.

**Do cuttings taken from *ETR1-1* plants form normal adventitious roots?**

No – this is the main reason for both this and last year's grant proposal. We have found no horticultural solutions to the inhibition in adventitious root formation on cuttings taken from *CaMV35S-ETR1-1* plants. We have produced putative plants that produce long lasting flowers as a result of carrying the AP1-NR construct. Currently we breeding these primary transgenic plants to see if the phenotype can be passed onto progeny – we are also making additional plants with the FBP1-NR construct. This work is described below as part of this funding request for the final year of this project.

**Does ethylene sensitivity affect pollen viability and pollen tube growth?**

Since we have inbred ETR1-1 and wild-type lines available, we have been investigating ethylene's role in sexual reproduction by both the male and female gametophytes. By making reciprocal F1 hybrid and self-pollinations, we can now separate ethylene's role in paternal and maternal factors associated with reproduction. Using various *in vitro* pollen viability staining procedures, we have determined that pollen produced on ethylene insensitive plants does not show reduced viability compared to wild type pollen. We have also determined by fluorescence

microscopy that pollen tube growth *in vivo* is not affected by ethylene insensitivity after self or cross-pollination. With these observations we can conclude that any role that ethylene may play in development of the male gametophyte will likely not influence performance of the pollen in horticultural terms.

**Does ethylene sensitivity affect seed production and seed germination?**

From previous experiments, we knew that fruit produced on ethylene insensitive plants ripened slower, and that subsequent germination of seeds was reduced. By producing seeds made from self and reciprocal F1 hybrid crosses, we wanted to determine if ethylene insensitivity of the male or female gametophyte would affect seed production and subsequent seed germination. It is now clear to us that seed yield in terms of number of seeds produced is not affected by ethylene insensitivity. However, we have found that seeds produced on ethylene insensitive maternal plants have a significantly reduced seed weight. We have also determined that seeds produced on ethylene insensitive maternal plants germinate slower, and are generally weaker at early seedling growth stages. It should be noted that seed weight and germination of F1 hybrid seeds produced from using *ETR1-1* plants as the pollen parent are the same as those produced from self-pollinating wild-type plants. Therefore, reduced seed weight and germination rate of F1 hybrid seeds does not result directly from ethylene insensitivity, rather, it results from processes associated with having delayed ripening in ethylene insensitive maternal fruit. Since the ETR1-1 transgene acts in dominant Mendelian fashion, it is logical to think that crosses to produce ethylene insensitive F1 hybrid plants will have to be made using a wild type maternal parent and an ethylene insensitive male parent.

In conducting all of this research, we discovered that there are obvious benefits and problems that arise from making a plant completely insensitive to ethylene. Since these problems could have serious implications for how this technology is to be used in the floriculture industry, we felt there was a need to engineer ethylene insensitivity only in specific parts of the plant – i.e., ethylene insensitivity in the flower only. Based on information obtained in this work, we proposed one objective last year to complete this project:

**Objective 4 - Devise methods to engineer tissue specific ethylene insensitivity.**

In order to circumvent the problems associated with inhibition of adventitious root formation due to ethylene insensitivity, graduate student Donna Clevenger engineered petunia plants with a new genetic construct that will allow expression of the mutant *ETR1-1* gene in floral tissues only. Using a promoter from the *Apetela 1* gene from *Arabidopsis*, we tried to confer ethylene insensitivity only at flowering and only in floral tissues. This gene has been shown to control some of the early events in flower initiation of *Arabidopsis* and the promoter (*AP1*) is known to

drive expression of genes specifically in floral tissues. We made over 75 confirmed transformations of this gene into two separate inbred petunia genetic backgrounds, but we only discovered one putative transgenic plant showing delayed floral senescence. This plant was self-pollinated and progeny were compared to both wild type and previously engineered *CaMV35S-ETR1* plants to determine if the plants displayed a delayed wilting phenotype. Unfortunately, we did not find that ethylene insensitivity was conferred in our progeny trials of plants containing the *Apetala1-ETR1-1* transgene. To back up this approach, we engineered another flower specific ethylene insensitive construct into petunia. Using the flower specific petunia promoter *FBP1* combined with the mutant *ETR1-1* gene we were able to obtain three primary transformants with putative flower specific ethylene insensitivity. At the current time, we have trials of progeny produced from these plants that are close to flowering, and they should help us determine whether flower specific ethylene insensitivity is achievable using this approach. Since this promoter is native in the petunia plant, we are hoping that we will be able to produce higher levels of *ETR1-1* expression in flowers than could be obtained using the *AP1* promoter from *Arabidopsis thaliana*.

At this point, we feel we have successfully completed the last year of this project using the genetic tools currently available to us. This work is crucial to providing the molecular genetics tools needed to continue making progress in this rapidly progressing area of research.