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# ETHYLENE INVOLVEMENT IN STRESS RESPONSES OF HORTICULTURAL CROPS

A Thesis in

Horticulture

by

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# Chapter 1

## Introduction and Literature Review

### INTRODUCTION

The gaseous plant hormone ethylene is known to be involved in the regulation of various plant developmental processes including seed germination, leaf and flower senescence, fruit ripening, and organ abscission (Abeles et al., 1992). Environmental stresses can induce ethylene biosynthesis. Stress induced ethylene elicits adaptive responses in plant development, which can be exemplified by the formation of adventitious roots and aerenchyma formation in plants grown under water-logging or nutrient starvation conditions, and by the rapid stem elongation in submerged semiaquatic plants. Wounding of tissues caused by insect or pathogen attack stimulates ethylene and plant defense mechanisms. Abscising plant organs in response to adverse environmental conditions ensures reproductive success and fruit set, facilitating dispersal of seeds and removal of organs that are no longer needed.

The ethylene biosynthetic pathway has been determined, and tremendous progress has been made in understanding ethylene signal transduction by chemical and genetic inhibition of signal perception and manipulation of the ethylene biosynthetic genes in transgenic crops. The alteration of ethylene synthesis, perception or response might alleviate or eliminate undesirable effects of ethylene, prompting delay of flower

senescence, fruit ripening and organ abscission, and improving postharvest or postproduction quality. However, global interference in ethylene synthesis or action could interfere with important ethylene-mediated responses such as seed germination, plant growth and development, and adaptive plant responses to unfavorable environmental conditions. Therefore, we posed several questions concerning the role of ethylene on overall plant growth responses.

In the second chapter, we investigate the effects of genetic inhibition of ethylene sensitivity on general plant growth responses to phosphorus deficiency. By utilizing two species impaired in ethylene responsiveness, we were able to determine the involvement of ethylene on morphological and physiological aspects of plant responses to phosphorus deficiency.

In the third chapter, we focus on ethylene involvement in both biotic and abiotic stresses by imposing low phosphorus and pathogen treatments. The strong stimulation of ethylene production is a common characteristic of plants infected by certain pathogens. Assuming that genetically inhibited ethylene sensitivity is associated with disease resistance and susceptibility, we investigate black root rot disease development symptoms in ethylene insensitive petunia under low phosphorus stress.

In the fourth chapter, we investigate ethylene-induced petal abscission that occurs during shipping stress, which is a significant problem in Regal Pelargonium (*Pelargonium* x*domesticum*). The Penn State plant breeding program has resulted in the development of Regal Pelargonium genotypes with exceptional postharvest characteristics. Therefore, we investigated the mechanism by which these genotypes have delayed petal abscission. Further, in an attempt to induce better postproduction quality,

we studied the effect of the ethylene action inhibitor (1-methylecyclopropane; 1-MCP) on the prevention of petal abscission during shipping.

## LITERATURE REVIEW

### Ethylene biosynthesis pathway

The amino acid methionine (Met) is the precursor of ethylene. The formation of S-adenosyl-methionine (SAM) is catalyzed by SAM synthetase from methionine at the expense of ATP utilization. SAM is converted by ACC synthase (ACS) to 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene (Adams and Yang, 1979) (Figure 1.1). In addition, ACS also produces 5'-methylthioadenosine (MTA) which is then converted to methionine by the Yang Cycle. Therefore, ethylene can be synthesized continuously without demanding an increasing pool of methionine (Wang et al., 2002). ACC oxidase (ACO) catalyses the final step of ethylene biosynthesis using ACC to generate ethylene, $CO_2$, and cyanide, which is detoxified to β-cyanoalanine by β-cyanoalanine synthase (β-CAS) to prevent toxicity of accumulated cyanide during high rate of ethylene synthesis.

The limiting step in ethylene biosynthesis is the conversion of SAM to ACC catalyzed by ACS (Kende, 1993). ACS is encoded by a multigene family containing at least seven genes in *Arabidopsis* (Arteca and Arteca, 1999; Liang et al., 1992; Samach et al., 2000) and at least ten genes in tomato (*Lycopersicon esculentum*) (Barry et al., 2000). The expression of ACS is highly regulated and each is induced in a different way by

developmental or environmental cues such as wounding, auxin, pathogen attack, drought, hypoxia, and ozone (Abel et al., 1995). Both positive and negative feedback regulation of ethylene biosynthesis have been reported in different plant species (Barry et al., 2000 ; Kende, 1993; Nakatsuka et al., 1998). ACS enzymes are spatially and temporally regulated at the transcriptional level. ACO is also somewhat regulated and ethylene inducible. One of the four genes appears to encode most of the ACO in tomato fruits.

Alternatively, ACC can also be converted to malonyl-ACC (MACC) by ACC-N-malonyltransferase in conjugation with malonate (Hoffman et al., 1982). MACC does not break down and conjugation of ACC takes place in the cytoplasm and the product MACC is stored in the vacuole (Bouzayen et al., 1989).

**Ethylene perception and signaling transduction pathway**

There are five ethylene receptors in *Arabidopsis*, ERT1, ETR2, EIN4, ERS1, and ERS2 (Figure 1.2). The receptor family can be divided into two subfamilies based on structural similarities (Bleecker, 1999). The ETR1-like subfamily, including ETR1 and ERS1, is characterized by having three hydrophobic subdomains at the N-terminus and a conserved histidine-kinase domain. The ETR2-like subfamily, composed of ETR2, ERS2 and EIN4, shows additional hydrophobic extension at the N-terminus, and carries degenerate histidine-kinase domains lacking of one or more elements necessary for catalytic activity. ERS is 67% identical to ETR1 at the amino acid level, but lacks the receiver domain of the response regulator. ERS also acts as an ethylene receptor, partially redundant in function with ETR1. In tomato the *Never Ripe* (*NR*) gene encodes a receptor

with a high similarity to the *Arabidopsis* ETR1 class genes without a receiver domain, while LeETR1 is similar to *Arabidopsis* ETR2 class genes with a receiver domain.

The ETR1 gene encodes an ethylene receptor. The N-terminal domain contains three hydrophobic transmembrane segments, which are the most conserved regions among the ETR1 homologs and required for ethylene binding. The C-terminal portion of ETR1 is homologous to the histidine kinases and response regulator domains of bacterial two component signal transduction systems (Chang et al., 1993 ). The sensor is responsible for perceiving the signal, inducing autophosphorylation of the histidine kinase domain on a conserved histidine residue. Ethylene binding occurs at the N-terminal transmembrane domain of the receptors, and requires copper as a co-factor (Rodriguez et al., 1999; Schaller and Bleecker, 1995). RAN1, a copper transporter, is involved in delivery of copper to the ethylene receptor (Hirayama et al., 1999).

Mutations at the CTR1 locus result in a constitutive ethylene sensitive phenotype indicating that CTR1 negatively regulates the downstream signaling events (Kieber et al., 1993). The amino acid sequence of CTR1 revealed that the gene codes for a serine or threonine protein kinase related to the mammalian RAF kinases that initiate MAP kinase cascades (Bleecker, 1999). EIN2 acts downstream of CTR1 and upstream of EIN3. The EIN2 gene encodes a novel integral membrane protein probably involved in transducing he signal to the nucleus (Alonso et al., 1999). Mutations in EIN2 result in complete loss of ethylene responsiveness throughout plant development. The EIN3 gene encodes a nuclear-localized protein that belongs to a multigene family in *Arabidopsis*.

In the absence of an ethylene signal, ethylene receptors activate a Raf-like kinase, CTR1, and CTR1 in turn negatively regulates the downstream ethylene response

pathway, possibly through a MAP-kinase cascade. Binding of ethylene inactivates the receptors, resulting in deactivation of CTR1, which allows EIN2 to function as a positive regulator of the ethylene signal transduction pathway. EIN2 positively signals downstream to the EIN3 family of transcription factors located in the nucleus. EIN3 binds to the promoter of ethylene responsive genes and activates their transcription in an ethylene-dependent manner.

## Manipulation of the ethylene signal transduction pathway

### *Genetic approaches to reducing ethylene response*

Ethylene insensitive mutants were identified by seedling bioassays using the lack of normal ethylene-mediated inhibition of hypocotyl elongation in dark-grown seedlings as a screen (Bleecker et al., 1988; Guzman and Ecker, 1990). Four dominant mutant alleles of the ETR1 gene have been identified, and all of them involve single amino acid changes within the hydrophobic N-terminal domain of the encoded protein (Bleecker and Schaller, 1996). The *etr1-1* and *etr1-4* alleles completely eliminate responsiveness to ethylene, whereas the *etr1-2* and *etr1-3* alleles display reduced responsiveness to ethylene. The *etr1-1* mutant in *Arabidopsis thaliana* confers dominant and nearly complete insensitivity. Transgenic plants expressing *etr1-1* were constructed with the constitutive CaMV-35S promoter in tomato, petunia, and tobacco plants (Wilkinson et al., 1997). Transgenic *etr1-1* petunia exhibited delayed flower senescence and fruit ripening, and strong reduction in adventitious root formation (Clark et al., 1999;

Wilkinson et al., 1997), and the flowers failed to senesce following exogenous ethylene treatment and pollination (Wilkinson et al., 1997).

The *Nr* mutation of tomato is a dominant single gene mutation which confers ethylene insensitivity in seedlings and flowers, and impaired color change and softening in fruits (Lanahan et al., 1994; Wilkinson et al., 1995). Like ERS, the predicted NR protein lacks the response regulator domain. In *Nr*, a mutation of the ethylene receptor encoded by the NR gene, leads to the loss of the ability to bind ethylene (Hackett et al., 2000 ; Lanahan et al., 1994; Wilkinson et al., 1995). Dosage response showed that *Nr* tomato seedlings retain some residual sensitivity in the presence of exogenous ethylene (Yen et al., 1995).

*Chemical approaches to reducing ethylene response*

Inhibitors of each step in the ethylene biosynthetic pathway were elucidated. ACC synthase, a pyridoxal phosphate-dependent enzyme, is inhibited by aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA) (Baker et al., 1977). The conversion of ACC to ethylene can be inhibited by aminoisobutyric acid (AIBA), and structural analogues of ACC (Satoh and Esashi, 1983). Other inhibitors of ACO include cobalt ions, n-propyl gallate, sodium caprylate, benzylisothiocyanate, fatty acids, high phosphate concentrations, salicylic acid and chilling temperatures (Abeles et al., 1992). Inhibitors of ACS and ACO only prevent ethylene biosynthesis, giving little protection in an ethylene-polluted environment. Other compounds such as carbon dioxide, silver thiosulfate (STS), 2,5-norbornadiene (2,5-NBD) and diazocyclopentadiene (DACP) are effective ethylene action inhibitors (Blankenship and Dole, 2003).

1-methylcyclopropene (1-MCP) has been developed as an inhibitor of ethylene action. 1-MCP binds to ethylene binding sites with 10 times higher affinity than that of ethylene (Sisler and Serek, 1997), and therefore, is active at a lower concentration than ethylene. In a formulation developed for commercial use, the gaseous form of 1-MCP is caged in dextran and released from EthylBloc powder when mixed with water. The compound is used at low rates, and non-phytotoxic at concentrations much higher than those needed for maximum effectiveness (Serek et al., 1994; Sisler et al., 1996). 1-MCP protects plants from both endogenous and exogenous sources of ethylene (Blankenship and Dole, 2003). It efficiently prevented flower abscission in various flowering potted plants (Serek and Sisler, 2001; Serek et al., 1994) as effectively as STS. However, 1-MCP had no effect on normal senescence in the absence of exogenous ethylene (Serek and Sisler, 2001; Serek et al., 1994) and the effect was transitory since abscission rate increased with time after the application (Cameron and Reid, 2001). Therefore, alternative methods are still needed to effectively control ethylene action.

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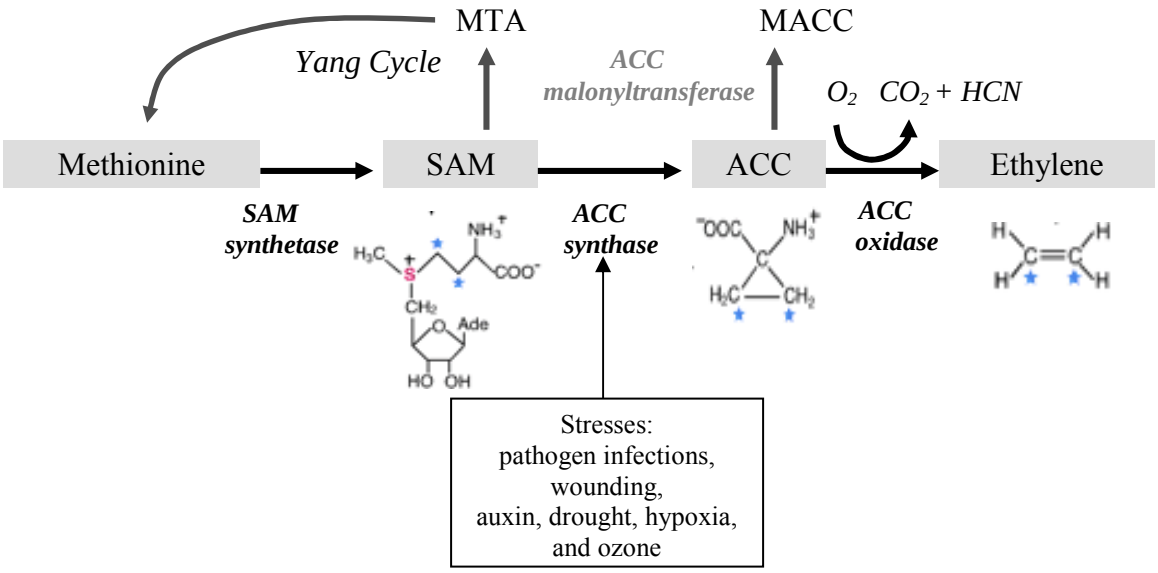


Figure 1.1. The ethylene biosynthetic pathway. The formation of *S*-AdoMet is catalyzed by SAM synthetase from the methionine. The conversion of SAM to ACC is catalyzed by ACC synthase which is the rate-limiting step of ethylene synthesis under most conditions. The by-product MTA generated during ACC production by ACC synthase is recycled through Yang cycle. The conversion of ACC to ethylene is catalyzed by ACC oxidase, generating carbon dioxide and cyanide. (Adapted from Figure. 1 in Wang et al. (2002)).

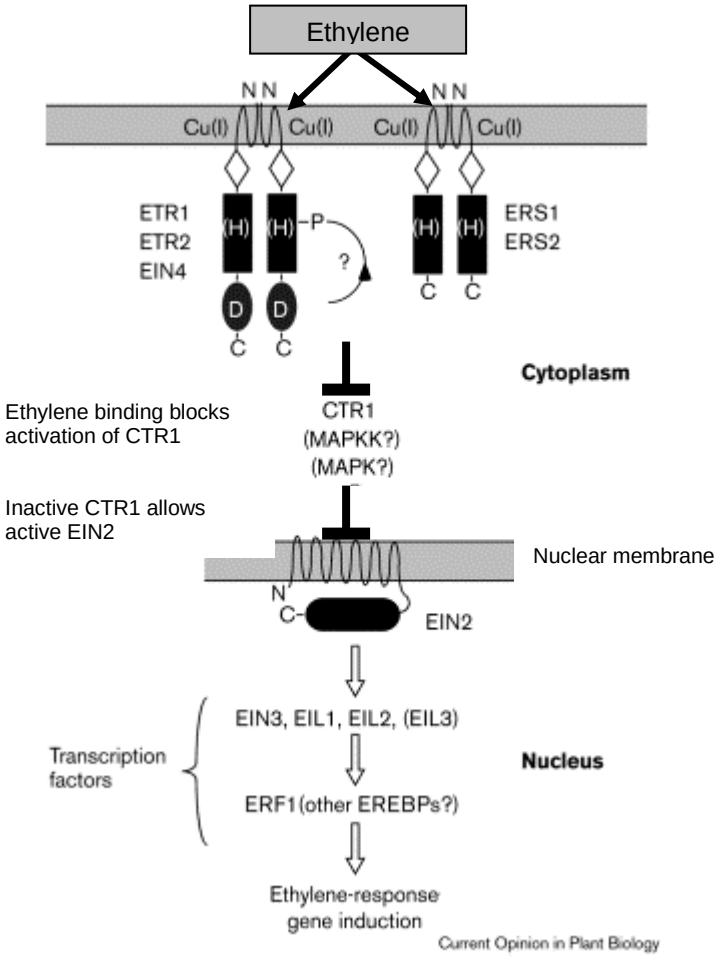


Figure 1.2. A schematic diagram of the ethylene signal transduction pathway. Ethylene is perceived by ethylene receptors (ETR1, ERS1, ETR2, EIN4 and ERS2) localized on the cell membrane. In the absence of ethylene, the receptors activate the downstream negative regulator CTR1. Ethylene binding inhibits receptor activation of CTR1, thus permitting ethylene response gene induction in target tissues. (Adapted from Figure. 1 in Chang and Shockey (1999))

# Chapter 2

## The effects of ethylene insensitivity on growth responses to phosphorus deficiency in tomato and petunia


## ABSTRACT

*Nr* (never-ripe) tomato and *etr1-1* (ethylene-resistant) petunia plants were evaluated to investigate the effect of ethylene on root and shoot development in response to low phosphorus stress. Adventitious root formation in tomato plants was significantly increased by low phosphorus in wild-type, but not in *Nr*. Ethylene production by adventitious roots was reduced by low phosphorus in both genotypes. The results suggest that enhanced adventitious root formation in phosphorus deficient wild-type tomato was induced by the increase in tissue sensitivity to ethylene, and that ethylene perception plays an important role in carbon allocation to adventitious roots under low phosphorus stress. Shoot growth of both species was decreased by low phosphorus similarly in ethylene sensitive and resistant genotypes. Reduced ethylene sensitivity of *Nr* tomato did not affect most plant growth responses to low phosphorus, while ethylene insensitivity of *etr1-1* petunia significantly reduced shoot weight, root weight, and total biomass at 4 weeks after transplanting, but not at 7 weeks regardless of phosphorus level. In tomato, the effective quantum yield of photosystem II was affected by phosphorus level at 2

weeks after transplanting but not by genotype. However, that of *etr1-1* petunia grown with low phosphorus was significantly lower than wild-type at 2 and 4 weeks after transplanting, and sequentially recovered to the level of the wild-type, suggesting that *etr1-1* petunia under low phosphorus following transplanting may undergo more severe stress compared to *Nr* tomato due to the stronger constitutive ethylene insensitivity. Our results demonstrate that ethylene mediates adventitious root formation in response to phosphorus stress and plays an important role for quick recovery of plants imposed to multiple environmental stresses, i.e. transplanting and low phosphorus.

## INTRODUCTION

Low phosphorus availability is a primary constraint to optimal plant growth because of its low mobility and spatial variation in soils (Lynch and Deikman, 1998). The ability of roots to explore the soil for phosphorus and to acquire it efficiently is an important component of plant adaptation to low phosphorus availability (Lynch and Ho, 2004). Although it is known that plants display various adaptive responses to low phosphorus availability, including physiological or morphological root characteristics which may affect phosphorus acquisition (Lynch and Brown, 2001; Raghothama, 1999), it is not clear how root growth responses to low phosphorus availability are mediated.

Plant hormones play an important role as mediators in plant adaptation responses to environmental signals (Voesenek and Blom, 1996). Ethylene is involved in root responses to edaphic stresses such as nutrient deficiencies and toxicities (Lynch and Brown, 1997), and has been proposed to be involved in root elongation, lateral and

adventitious root formation, root extension, radial expansion, root hair production and aerenchyma formation (Borch et al., 1999; Drew et al., 1979; Drew and Saker, 1978; Fan et al., 2003; He et al., 1992; Konings and Jackson, 1979; Lynch and Brown, 1997; Lynch and Brown, 2001; Tadeo et al., 1997; Visser et al., 1997; Zhang et al., 2003).

Root responses to edaphic stresses appear to be promoted by changes in ethylene synthesis and/or responsiveness. The responses of cucumber roots to iron deficiency was promoted by the ethylene precursor, 1-aminocyclopropane-1-carboxylate (ACC), but inhibited by ethylene synthesis or action inhibitor (Romera and Alcantara, 1994). Later stages of low phosphorus-enhanced root hair development in *Arabidopsis* are regulated by ethylene (Zhang et al., 2003). Ethylene production (with aminoethoxyvinyl-glycine) or action (with 1-methylcyclopropene) inhibitors increased primary root elongation of *Arabidopsis* in high phosphorus and decreased it in low phosphorus (Ma et al., 2003). Phosphorus deficient roots of common bean plants produced twice as much ethylene per dry weight as phosphorus sufficient roots (Borch et al., 1997).

In contrast, nitrogen or phosphorus starved adventitious roots of maize produced consistently reduced rates of ethylene (Drew et al., 1989 ), which substantially enhanced the sensitivity of ethylene-responsive cortical cells increasing aerenchyma formation (He et al., 1992). Altered ethylene responsiveness seems also to be involved in adventitious root responses to waterlogging. Inhibiting ethylene perception with silver prevented adventitious root development in soybean (*Glycine max*) acclimatized to flooding (Bacanamwo and Purcell, 1999). These results suggest that ethylene is involved in adventitious root formation directly or via altered tissue sensitivity under low phosphorus availability.

In this study, we investigate the role of ethylene in mediating responses to phosphorus stress using two mutants with impaired ethylene sensitivity. The *Nr* mutation is a dominant single gene mutation in tomato (*Lycopersicon esculentum*) which confers ethylene insensitivity in seedlings and flowers, and impaired color change and softening in fruits (Lanahan et al., 1994; Wilkinson et al., 1995). The mutation of the ethylene receptor encoded by the NR gene leads to the loss of the ability to bind ethylene (Hackett et al., 2000 ; Lanahan et al., 1994; Wilkinson et al., 1995). However, dosage response demonstrated that *Nr* tomato seedlings retain residual sensitivity in the presence of 1 μL $L^{-1}$ exogenous ethylene in both root and shoot (Yen et al., 1995). Unlike *Nr* tomato, the *etr1-1* mutant in *Arabidopsis thaliana* displays nearly complete insensitivity to as high as 100 μL $L^{-1}$ ethylene (Bleecker et al., 1988). An ethylene insensitive petunia was generated by transforming 'Mitchell Diploid' petunia with the constitutive CaMV-35S promoter driving expression of the dominant mutant *etr1-1* gene (Wilkinson et al., 1997). Transgenic *etr1-1* petunia is reported to exhibit delayed flower senescence and fruit ripening (Wilkinson et al., 1997), and the flowers failed to senesce following exogenous ethylene treatment or pollination (Wilkinson et al., 1997). Clark et al (1999) observed different rooting responses to ACC of *Nr* and *etr1-1* cuttings. Adventitious rooting was not completely inhibited in *Nr* cuttings and was promoted by the application of ACC, while the rooting of *etr1-1* cuttings was almost completely inhibited, supporting the hypothesis that the *etr1-1* petunia is more ethylene insensitive than *Nr* tomato. However, it has not been demonstrated how the differences in ethylene insensitivity of both species could lead to the differences in adventitious rooting and plant performance under low phosphorus stress. Low phosphorus is associated with enhanced adventitious rooting

(Miller et al., 2003). We hypothesized that these genotypes perform differently under low phosphorus stress and during normal production protocols that include seedling establishment and transplanting.

The objective of this study was to examine the effects of ethylene insensitivity on root formation as well as growth responses of tomato and petunia roots and shoots to phosphorus deficiency. In this study, it is demonstrated that ethylene sensitivity is required for tomato plants to adapt their root morphology in response to low phosphorus availability. Furthermore, we suggest that ethylene sensitivity is necessary for the prompt recovery from transplant stress in petunia when low phosphorus stress is also applied.

## MATERIALS AND METHODS

### Plant materials

Inbred 'Pearson' (wild-type) and near-isogenic mutant *Never-ripe* (*Nr*) tomato (*Lycopersicon esculentum* Mill.), and inbred 'Mitchell Diploid' (wild-type) and transgenic line 44568 (Wilkinson et al., 1997) *etr1-1* petunia (*Petunia* x*hybrida*) seeds were obtained from Dr. David G. Clark at the University of Florida. Tomato seeds of both genotypes were germinated in an 8×15 plug tray filled with Aggregate Plus Media – Sunshine Mix-4 containing starter fertilizer (SUN GRO Horticulture, Bellevue, Washington) and placed in a greenhouse under intermittent mist with tap water. Three to four week-old seedlings were transplanted into containers as described below and grown in a greenhouse at University Park, PA, USA (40.79º N, 77.86º W) without supplemental irradiance. Day and night temperatures in the greenhouse during the experimental period

were 28 ºC ± 4 and 23 ºC ± 2, respectively. Relative humidity was 55% ± 5 and 64% ± 5 (day/night). Photosynthetically active radiation varied throughout the day with a maximum of 1500 μmol $m^{-2}s^{-1}$.

Petunia seeds were soaked in 100 $\mu l \cdot l^{-1}$ Gibberellic Acid ($GA_3$) overnight and rinsed thoroughly with distilled water before planting to promote germination. They were germinated in a 16×8 plug tray, and grown with similar cultural and environmental conditions as described for tomato. The experiments with tomato and petunia were conducted at the same times which were from April to June 2002 and from August to October 2002.

**Plant culture methods**

Established tomato or petunia seedlings were transplanted into 4 L pots filled with solid medium (40:40:20 v/v/v = vermiculite:perlite:sand) containing 1.5% (w/v) solid-phase phosphorus buffered alumina-P (Lynch et al., 1990) providing two desorption concentrations, low P (1 μM) and high P (100 μM), allowing a constant but regulated supply of phosphorus. Pots were automatically irrigated once a day while the plants were small, and twice a day as the plants grew older, with nutrient solution of pH 6.2-6.5 containing (in μM) 1500 $KNO_3$, 1200 $Ca(NO_3)_2$, 400 $NH_4NO_3$, 25 $MgCl_2$, 5 Fe-EDTA, 500 $MgSO_4$, 300 $K_2SO_4$, 300 $(NH_4)_2SO_4$, 1.5 $MnSO_4$, 1.5 $ZnSO_4$, 0.5 $CuSO_4$, 0.15 $(NH_4)_6Mo_7O_{24}$ and 0.5 $Na_2B_4O_7$. High and low phosphorus treatments contained 100 μM and 1 μM $KH_2PO_4$, respectively. In order to promote adventitious root growth in the hypocotyls, seedlings were planted at a length of hypocotyls of 2-3 cm below the medium surface and the pot was covered with a white plastic mat with a slit.

To investigate plant root growth responses without mechanical impedance, plants were grown in hydroponic culture in a greenhouse maintained as described above without supplemental light. For tomato plants, seedlings were germinated and grown in medium as described above. At the time of transplanting, the roots were thoroughly cleaned with distilled water and plants were transferred to hydroponic containers (size: 51 × 36 × 22 cm) filled with 30 L complete nutrient solution as described above containing soluble phosphorus as well as 1.5% (w/v) solid-phase buffered alumina-P providing two different phosphorus desorption rates, low P (1 μM) and high P (100 μM). Nutrient solutions were replaced weekly. Solution pH was monitored every two days and adjusted to between 6.2 and 6.5 with 1 M NaOH as needed. Six plants were grown per container. Containers were kept fully oxygenated by bubbling with air.

For petunia, the roots of 3 to 4 week old seedlings were rinsed carefully with distilled water and transferred to hydroponic containers (size: 31 × 22 × 21 cm) filled with 10 L complete nutrient solution. The nutrient regime was the same as described for tomato. Three plants were grown per container.

**Ethylene production**

To measure ethylene production in shoots, subsamples were collected from the main stem consisting of 3 to 4 leaves for tomato, and 7 to 8 leaves for petunia including the apical meristem. Ethylene production in adventitious roots and other roots were separately determined in tomato. Roots were separated from shoots, thoroughly rinsed with distilled water, and adventitious roots were removed with a razor blade and placed

immediately into a 500 $cm^3$ glass jar for 1 h at 22 ± 1 ºC. For the ethylene measurement of the other roots, whole roots were used for the measurement without taking subsamples.

Ethylene was sampled with 1 $cm^3$ syringes from the head space of the sealed container and the concentration was determined by gas chromatography (GC) (Hewlett-Packard 6890, Palo Alto, CA, USA) fitted with a flame ionization detector and an activated alumina column. The detection limit was 0.013 $cm^3$ $m^{-3}$. Ethylene production (nl/g FW/h) was calculated on the basis of fresh weight of root and shoot samples. Sample fresh weight was measured immediately after the ethylene measurement and the sample was dried at 65ºC for 5 days to measure total dry weight of shoot or root.

**Photosynthesis and Chlorophyll fluorescence**

Chlorophyll fluorescence, $CO_2$ exchange rate (CER), and electron transport rate (ETR) were measured using the LiCor-6400 gas exchange system with a 6400-40 leaf chamber fluorometer (Li-Cor, Lincoln, NE, USA). Irradiance of 1500 μmol $m^{-2}s^{-1}$ PAR was provided by the 6400-40 LED light source. The measurements were performed under the ambient greenhouse conditions with 370 μmol $mol^{-1}$ of baseline leaf and reference chamber $CO_2$ concentrations adjusted by the LiCor-6400 $CO_2$ regulator.

The following parameters were determined when dF/dt (the rate of change in the fluorescence signal) approached zero and $CO_2$ concentrations in the leaf chamber reached a steady state (60-90 seconds): the effective quantum yield of PSII [$\Phi_{PSII} = (F'_m - F'_s)/ F'_m$], the electron transport rate ($ETR = \Phi_{PSII} \times PPFDa \times 0.5$), where $F'_m$ and $F'_s$ are maximum and steady-state fluorescence in the light–adapted measurement, respectively.

Fluorescence measurements were performed using the saturation pulse method concomitantly with the gas exchange measurements on the same leaves.

Young fully expanded, attached leaves of tomato or petunia plants were utilized for the measurements. Data were collected at least once every week between 11 am and 3 pm during the experimental period.

**Plant growth and development**

Plants grown in solid medium were harvested at 3 and 6 weeks after transplanting for tomato, and 4 and 7 weeks for petunia. Plants grown in hydroponic culture were harvested at 3 weeks after transplanting in tomato, and 3 and 6 weeks in petunia since the plants grew more vigorously in hydroponics compared to solid medium. Plant height, width, branch number (longer than 5cm), and leaf number (longer than 3 cm) were monitored weekly in both cultural systems. Plant width was calculated as an average of values from two perpendicular measurements. At the time of harvest, plant height, width, branch number, and leaf number were recorded again. Individual leaves were destructively harvested to measure leaf area with LI-3100 Leaf Area Meter (Glen Spectra Ltd., Stanmore, U.K.). Dry matter was determined from plant material dried at 65ºC for 5 days.

**Root system analyses**

Roots were harvested and thoroughly rinsed with distilled water. Adventitious roots, if any, were collected separately from the rest of the root system. A representative root subsample was collected when the root system was too large to be scanned for the

analysis of total root length. At least 2 days prior to scanning the root images with a flat-bed scanner (HP Scanjet IIc; Hewlett Packard, San Jose, Calif.), roots were stained with 0.16 % neutral red dye (Sigma Chemical Co., St. Louis, MO, USA) to create optimal contrast. Root length, root diameter, and surface area were estimated using WinRHIZO Pro software (Regent Instrument Inc. Quebec City, Quebec, Canada). The total root length was estimated by multiplying the subsample length by the dry weight ratio of the scanned subsample and the total dry weight of the root. All adventitious roots were stained and analyzed for adventitious root length as described above without taking a subsample. Specific root length [SRL, root length per unit root dry mass (m/g)] was calculated by dividing the total root length by the root DW. Dry matter was determined by drying the plant material at 65ºC for 5 days.

**Allometric analyses**

Dry matter of shoot, root, and adventitious roots of tomato plants harvested at 3 or 6 weeks after transplanting were used to analyze allometric relationship of root:shoot and adventitious root:total root. The allometric relationship was fitted by linear regression and the allometric coefficient (K) was obtained from the slope of the linear regression line (Hunt, 1990).

**Statistical analysis**

Data were analyzed using Minitab (Minitab Inc., State College, PA, USA). When the data were not normally distributed, the data were transformed with Box-Cox transformation, allowing data to be normally distributed. Then they were analyzed with

ANOVA general linear model. Arcsine transformation was conducted for analyzing the proportion of roots as adventitious roots. One-way analysis of variance was conducted with Tukey's multiple comparison method when phosphorus level or genotype had significant effects. Each experiment consisted of 2 genotypes and 2 phosphorus levels. Block effect was not included in the data output since we observed consistent results from the two repeated experiments. Therefore, the data were pooled and phosphorus and genotype effects were tested at $P < 0.05$. Repeated measures ANOVA of Fv/Fm, CER and ETR were performed using StatView (SAS Institute INC., Cary, NC, USA) to analyze treatment effects over time.

## RESULTS

### Plant growth responses to phosphorus deficiency

Low phosphorus availability significantly reduced overall plant growth in both tomato (Tables 2.1 and 2.2) and petunia (Tables 2.3 and 2.4). Plant height, width, branch number, leaf number, and leaf area were significantly decreased by low phosphorus at 3 or 4 weeks after transplanting, and remained lower throughout the growth period regardless of the genotype. Phosphorus deficiency significantly decreased shoot dry weight in both species (Tables 2.1, 2.2, 2.3 and 2.4), but more strongly than the reduction in root dry weight especially at 6 or 7 weeks after transplanting, resulting in a proportional increase in the root-to-shoot ratio (Tables 2.2 and 2.4).

In tomato, lengths of adventitious roots and other roots were significantly reduced by low phosphorus (Table 2.1). However, average root diameter was not affected by

phosphorus deficiency (Table 2.1). Correspondingly, total root length in petunia was reduced by low phosphorus, but root diameter was not altered by the treatment at 7 weeks after transplanting (Table 2.4).

**Effects of ethylene insensitivity on growth responses to low phosphorus**

Although ethylene insensitivity did not affect most plant growth responses to low phosphorus availability in tomato plants (no significant phosphorus by genotype interaction, Tables 2.1 and 2.2), significant phosphorus and genotype interactions were observed for adventitious root formation. The dry weight of adventitious roots in *Nr* was equal to that of 'Pearson' tomato under high phosphorus (Figure 2.1. A), while the proportion of root dry weight as adventitious roots was significantly reduced in *Nr* but not in 'Pearson' (Figure 2.1. B). The number of adventitious roots was about the same among all the treatments at 3 weeks after transplanting, but it was notably increased by low phosphorus in 'Pearson' tomato at 6 weeks after transplanting (Figure 2.2). There was no genotype effect on other root-related traits such as total root length, surface area and volume in both adventitious roots and other roots (Table 2.1).

Consistent with the results from tomato, there was no interaction between phosphorus level and genotype on various measures of growth in petunia (Tables 2.3 and 2.4) except the number of internodes (Table 2.3). We did not observe adventitious root formation in petunia.

**Chlorophyll fluorescence**

The effective quantum yield of PS II (Fv/Fm) of both tomato and petunia plants was low after transplanting but dramatically increased within 14 days and declined towards the end of the experiment (Figure 2.3). Repeated measures ANOVA demonstrated that Fv/Fm changed over time and phosphorus was the only variable to affect the values in tomato (Table 2.5, Figure 2.3.A). The phosphorus effect was significant at 14 and 17 days after transplanting, but the difference between phosphorus treatments disappeared by 21 days (Figure 2.3.A). The values were maintained as high as 0.5 over the growth period regardless of the treatments. The Fv/Fm of petunia plants reached 0.5 more slowly than that of tomato plants (Figure 2.3.B). The value reached 0.5 within 21 days in plants grown with high phosphorus, and took even longer in plants with low phosphorus. The values of both MD and *etr1-1* genotypes grown with high phosphorus were parallel throughout the experiment. However, the value of *etr1-1* genotype with low phosphorus was significantly lower than that of MD at 10 days and 28 days after transplanting. Based on the analysis with repeated measures ANOVA, all the variables, phosphorus level, genotype and time, influenced Fv/Fm values in petunia plants (Table 2.5). There were significant interactions among the variables over time. Values of Fv/Fm declined in all treatments towards the end of the growth period. Carbon dioxide exchange rate (CER) and electron transport rate (ETR) displayed the same tendency as Fv/Fm in both tomato and petunia plants (Figure 2.3 and Table 2.5).

**Ethylene production**

Shoots of tomato plants grown with low phosphorus have greater production rates of ethylene compared to those with high phosphorus, although there were no genotype differences (Figure 2.4. A). Adventitious roots had a higher rate of ethylene production than other tissues examined (Figure 2.4. B). Unlike the shoot, adventitious roots had significantly decreased ethylene production when they were grown with low phosphorus (Figure 2.4. B). Ethylene production rates remained lower in roots and higher in shoots even in plants harvested at 7 weeks after transplanting (data not shown).

Similarly, shoots of petunia plants grown with low phosphorus tend to have greater production rates of ethylene compared to those with high phosphorus (Figure 2.4. C). Root ethylene production of MD petunia was significantly reduced by phosphorus deficiency (Figure 2.4. C).

**Ethylene effects on carbon allocation under low phosphorus**

Allometric analysis was conducted for tomato plants as an index of the balance of growth between root and shoot components at two harvest times. The root-shoot allometric coefficient was not different between two genotypes regardless of phosphorus level (Figure 2.5 and Table 2.10). However, the adventitious root-root allometric coefficient was significantly higher in 'Pearson' under low phosphorus compared to the coefficients of other treatments (Table 2.10), indicating that 'Pearson' allocated a larger fraction of carbohydrates preferentially to the adventitious roots under low phosphorus stress. There was a significant interaction between phosphorus level and genotype in the adventitious root-root allometric coefficient at $P < 0.1$.

**Ethylene effects on plant growth responses**

While ethylene insensitivity significantly increased plant height in tomato plants only at 3 after transplanting (Tables 2.1 and 2.2), it consistently reduced that of petunias at 4 and 7 weeks after transplanting (Tables 2.3 and 2.4). Branch number was the same in both petunia genotypes at 4 weeks after transplanting (Table 2.3), but was slightly higher in *etr1-1* at 7 weeks after transplanting (Table 2.4). Shoot and root dry weights were smaller in *etr1-1* compared to MD petunia at 4 weeks after transplanting, but there was no significant difference between genotypes at 7 weeks after transplanting. Internode length was significantly decreased by low phosphorus in *etr1-1* genotype (Table 2.4 and Figure 2.7) but there was no difference in internode number (Table 2.4). Table 2.6 illustrates the changes in plant volume, calculated on the basis of plant height and width. While plant volume of tomato plants was affected only by phosphorus, that of petunia plants was affected by both phosphorus and genotype. The plant volume of *etr1-1* petunia was decreased by low phosphorus at 3 and 5 weeks after transplanting compared to wild-type of the same phosphorus treatment.

**Effects of growth media on plant growth responses**

Plants displayed similar growth responses to low phosphorus in hydroponic culture and solid medium (Tables 2.1, 2.2, and 2.7 for tomato, Tables 2.3, 2.4, 2.8 and 2.9 for petunia). However, low phosphorus had more significant effects on plant growth in hydroponics for both tomato and petunia, inducing more severe growth depression (Tables 2.12 and 2.13).

In tomato, shoot and root dry weights of both genotypes were dramatically reduced by low phosphorus compared to that of the same age plants grown in solid medium, resulting in significant reduction in total biomass (Tables 2.11 and 2.12). Regardless of the type of growth medium and phosphorus level, *Nr* tomato had significantly higher total biomass than 'Pearson' tomato (Tables 2.11 and 2.12). In *Nr* tomato, the proportion of root mass as adventitious roots was significantly decreased by low phosphorus in solid medium, but increased in hydroponics (Tables 2.11 and 2.12). Low phosphorus increased specific root length higher in hydroponic culture (Figure 2.6).

The growth of MD petunia in hydroponic culture was severely reduced by low phosphorus, particularly in the *etr1-1* genotype (Table 2.11). The dramatic decrease in shoot dry weight in hydroponic culture proportionally increased the root-to-shoot ratio (Tables 2.11 and 2.13).

## DISCUSSION

### Genetic inhibition of ethylene sensitivity does not alter growth responses to phosphorus deficiency in tomato and petunia

Phosphorus deficiency significantly reduced plant growth in both tomato and petunia. In tomato plants, genotypic (ethylene sensitivity) differences in response to low phosphorus were only observed for adventitious root formation, but not other plant growth responses (Tables 2.1, 2.2 and 2.7). In petunia, low phosphorus significantly reduced overall plant growth responses in both genotypes, although it was worse in the ethylene insensitive genotype (Tables 2.3 and 2.4). Our results demonstrate that the

inhibition of ethylene responsiveness has a relatively minor effect on plant morphology and development when phosphorus is a limiting factor.

**Ethylene perception is required for enhanced adventitious root formation under phosphorus deficiency**

In our experiment, both 'Pearson' and *Nr* tomato plants produced below-ground adventitious roots at both phosphorus levels, but neither petunia genotype produced adventitious roots under any of our growth conditions. Clark et al (1999) observed that the cuttings of both species produced adventitious roots but ethylene insensitive genotypes of these species exhibited different rooting responses to ACC treatment. However, lack of adventitious root formation in intact petunia plants made it impossible to compare the rooting responses of both species to low phosphorus.

The proportion of root dry weight as adventitious roots was significantly higher in 'Pearson' compared to *Nr* mutant of tomato under low phosphorus availability ($P < 0.01$ in solid medium, $P < 0.12$ in hydroponics), and the number of adventitious roots was also increased in 'Pearson' tomato by low phosphorus (Tables 2.1, 2.2 and 2.7), indicating that low phosphorus increases biomass allocation to adventitious roots relative to other root classes, as reported for common bean (Miller et al., 2003). However, increased adventitious root formation in phosphorus-deficient plants was not associated with altered ethylene production. Contrary to the findings which showed that low phosphorus availability increased ethylene production in roots of 5 week old bean plants (Borch et al., 1997), ethylene production rate was significantly decreased in phosphorus deficient roots of both tomato and petunia. This discrepancy might derive from differences in

developmental stage, root types, plant species and growing systems (Abeles et al., 1992; Borch et al., 1997; Mollier and Pellerin, 1999).

It has not been demonstrated that ethylene production rates significantly vary among root types. Adventitious roots produced about ten times more ethylene than other roots of the same treatment regardless of phosphorus level and genotype (Figure 2.4). Variation in ethylene production among root types may induce different responses to low phosphorus. Borch et al (1999) observed that AVG inhibited main root growth but increased lateral root density of common bean grown under low phosphorus, while exogenous ethylene reversed the effects, indicating that the effect of ethylene on root length depends on the root type and on phosphorus nutrition. Although it was not demonstrated in this experiment how other root types respond to low phosphorus, it would be intriguing to investigate the differential role of ethylene on various root types.

Ethylene production in shoots or roots of ethylene insensitive genotypes was not significantly different from wild-types regardless of plant species (Figure 2.4) and age (data not shown). Low phosphorus did not affect ethylene production rate in ethylene insensitive genotypes relative to wild-types. When ethylene production was measured with excised leaves from wild-type and *etr* mutant of *Arabidopsis*, similar rates of ethylene was observed (Bleecker et al., 1988). However, when they were pretreated with ethylene, ethylene production rate was greatly reduced in the wild-type but not in *etr* genotype (Bleecker et al., 1988). Enhanced ethylene production was also observed in pollinated *etr1-1* petunia flowers (Wilkinson et al., 1997). These results indicate that feedback control of ethylene biosynthesis is disrupted in ethylene insensitive genotypes. Lack of such observation of ethylene insensitive genotypes in response to low

phosphorus suggests that low phosphorus may be a weaker inducer of ethylene biosynthesis than exogenous ethylene treatment or pollination in these genotypes.

The induction of aerenchyma in adventitious roots under hypoxia normally results from enhanced ethylene production and entrapment (Drew et al., 1979; Visser et al., 1996). In contrast, tomato and petunia in our experiments demonstrated equal or lower ethylene production by adventitious roots under low phosphorus. Reduction of ethylene production has been also reported in nitrogen or phosphorus-starved maize roots (Drew et al., 1989 ), and was associated with enhanced ethylene sensitivity and increased aerenchyma formation in adventitious roots (He et al., 1992). Enhanced adventitious root formation in phosphorus deficient 'Pearson' tomato could be induced by the increase in tissue sensitivity to ethylene, since *Nr* plants did not show the increase. The effects of the Nr gene demonstrate the importance of ethylene perception in adventitious root development. Adventitious roots may be advantageous for phosphorus acquisition by enhancing plant foraging in phosphorus-rich topsoil, reducing inter-root competition, and increasing the volume of soil to be explored by dispersing lateral roots (Miller et al., 2003). However, the temporal and spatial benefits of adventitious roots for phosphorus uptake (Miller et al., 2003) were not realized in overall plant growth improvement in this experiment, presumably due to uniform phosphorus distribution in the soilless medium.

Our results indicate that low phosphorus stress increased carbon (biomass) allocation to adventitious roots in tomato plants (Table 2.10). The higher allocation of biomass to adventitious roots was also demonstrated in common bean (Miller et al., 2003). This research was conducted with two solanaceous species with typical herbaceous dicot root systems. However, tomato roots are similar to common bean in

having a distinctive root system, while petunia roots are more fibrous without a distinguishable primary root. Due to the massive production of other roots in tomato plants, we could separate only adventitious roots from other roots. Higher formation of adventitious roots was accompanied by reduced root dry weight of other roots of 3 week old tomato plants in solid medium (Table 2.1). This result is consistent with the observation made by Clark et al. (1999), in which 35-day old 'Pearson' plants had less below ground root mass than *Nr* tomato plants but produced more adventitious roots at 77 days. These results suggest that there might be reduction in other root types to compensate for the construction cost of adventitious roots. Although adventitious roots have significantly lower metabolic construction cost per unit biomass of root length than basal roots (Miller et al., 2003), higher demand for carbon allocation to adventitious roots may cause the reduction of other root systems.

**Low phosphorus has stronger effects in hydroponic culture**

Tomato and petunia plants were grown in both hydroponic culture and solid medium to verify plant growth responses to ethylene insensitivity and low phosphorus in different growing systems. Low phosphorus had significantly stronger effects in hydroponic culture for both tomato (Tables 2.11 and 2.12 and Figure 2.6) and petunia (Tables 2.11 and 2.12). Such increased impact of low phosphorus in hydroponics could be partially caused by increase in competition for phosphorus since we grew six plants together in the same hydroponic container. We observed that plant height of low phosphorus was disproportionately decreased in hydroponics compared to that of plant grown with high phosphorus toward the end of the experiment (data not shown).

Therefore, as plants become larger, the competition for phosphorus uptake in hydroponics may lead to quicker deprivation of phosphorus in the container compared to the solid medium where each plant had access to the full volume (4 L) of the pot.

The proportion of adventitious roots and adventitious root length was increased by low phosphorus to a greater extent in hydroponics than in solid medium. Since adventitious rooting is influenced by numerous environmental factors, such as medium moisture and aeration near the hypocotyls and phosphorus stress (Miller et al., 2003), the enhanced adventitious root formation observed in hydroponics could be partly due to the higher moisture and greater phosphorus stress in the container. Similarly, hydroponics increased specific root length of both root types under low phosphorus (Figure 2.6). Increased access of roots to water has been associated with higher specific root length (Eissenstat, 1991). Therefore, higher water availability in combination with low phosphorus around the root system might have synergistically increased root diameter and production of lateral roots in hydroponics, resulting in higher specific root length.

**Ethylene perception is critical for quick recovery of plants from multiple stresses**

We observed significant retardation of plant growth in *etr1-1* petunia for several weeks after transplanting compared to MD, especially under low phosphorus availability, but no delay in plant growth was observed in *Nr* tomato. This was not a result of developmental differences in growth rate, since untransplanted *etr1-1* petunia seedlings grew continuously even after their leaves touched neighboring seedlings (*etr1-1* plants grew with a leaf angle of 43 ± 2 º from the soil surface, while leaves of MD petunia grew with a more upright angle of 57 ± 3 º). Further, internode number at 7 weeks after

transplanting was not different among the treatments, indicating that it does not account for the reduction in height. Thus, we speculated that the growth delay in *etr1-1* petunia after transplanting might be due to greater susceptibility to multiple stresses, in this case transplanting followed by low phosphorus stress.

The use of chlorophyll fluorescence has been introduced as an diagnostic tool for photoinhibitory stress (Krause and Weis, 1991). Stresses such as nutrient deficiency, metal toxicity and water deficit can lead to photo-oxidative stress in crop plants (Foyer et al., 1994). In healthy plants, the value of chlorophyll fluorescence is typically 0.5, and lower values indicate that a proportion of PSII reaction centers are damaged (Maxwell and Johnson, 2000). In order to test our hypothesis, we measured chlorophyll fluorescence as an indicator of photo-oxidative stress induced by transplanting accompanied by low phosphorus stress.

Our results showed that Fv/Fm of *Nr* tomato under low phosphorus quickly reached 0.5 after a slight reduction at 14 days after transplanting. However, the Fv/Fm value of *etr1-1* petunia was significantly decreased at 10 and 28 days after transplanting by low phosphorus availability compared to MD petunia of the same treatment, and the value did not reach 0.5 until 35 days after transplanting. These decreases in Fv/Fm of *etr1-1* petunia by low phosphorus were followed by decreases in plant growth (see volume, Table 2.6). Based on these observations, we concluded that *etr1-1* petunia under low phosphorus may undergo more severe stress compared to *Nr* tomato due to the stronger constitutive ethylene insensitivity, resulting in significant delay of plant recovery from multiple stresses. The different progress of Fv/Fm value between tomato and petunia may be indicative of genetic differences between the species for this parameter.

We speculate that the first stagnation of the Fv/Fm in *etr1-1* might be caused by transplant stress in addition to low phosphorus stress. The plants could have recovered from the transplant stress within 2 weeks but cumulative phosphorus stress might cause the second stagnation in Fv/Fm value. This interpretation is supported by the fact that when *etr1-1* genotypes were grown with only one stress (transplant stress), they could recover in 2 weeks without further stagnation in the Fv/Fm value (Figure 2.3. B). The decline of Fv/Fm in high phosphorus treatments at 7 weeks could be caused by plant ageing and severe root restriction, while that of low phosphorus might be due to shift from vegetative phase to reproductive phase and cumulative nutrient deficiency. Interestingly, both optimally grown *etr1-1* petunia and low phosphorus stressed MD petunia showed relatively higher values of Fv/Fm, suggesting that either lack of ethylene perception or low phosphorus stress imposed relatively weak stresses. These observations suggest that *etr1-1* genotype is not likely to perform properly under horticultural circumstances where plants can often encounter multiple stresses such as drought, darkness, mechanical stress, and nutrient deficiency.

Since ethylene is a hormone involved in various plant developmental processes and environmental stimuli, global interference in ethylene action could interfere with important ethylene-mediated responses such as seed germination, plant growth and development (Clark et al., 1999; Klee and Clark, 2002), and plant adaptation to unfavorable environmental conditions such as phosphorus deficiency (Lynch and Brown, 1997). Ethylene insensitive plants have increased disease susceptibility and failure of adventitious root formation from vegetative cuttings (Geraats et al., 2002; Klee and Clark, 2002; Knoester et al., 1998). In this study, we clearly demonstrated that ethylene

insensitive genotype of petunia does not perform well for several weeks after transplanting under low phosphorus. Therefore, we concluded that ethylene perception is important for plants to quickly recover when multiple environmental stresses are imposed on the plants.

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Table 2.1. The effect of phosphorus availability on root and shoot development in 'Pearson' and *Nr* tomatoes grown in solid medium with low phosphorus (1 μM) and high phosphorus (100 μM) and harvested at 3 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-Pearson | High P-*Nr* | Low P-Pearson | Low P-*Nr* | P | G | P*G |
| Plant height (cm) | 30.7 | 34.6 | 23.8 | 27.6 | 60.66 *** | 13.42 ** | 0.16 ns |
| Branch number | 1.9 | 2.1 | 1.0 | 1.0 | 11.02 *** | 0.19 ns | 0.19 ns |
| Leaf number | 14.0 | 14.6 | 7.9 | 10.0 | 9.69 ** | 0.68 ns | 0.71 ns |
| Leaf area ($cm^2$) | 847.6 | 915.4 | 501.9 | 375.5 | 61.39 *** | 0.19 ns | 1.63 ns |
| Specific leaf area ($mg/cm^2$) | 2.446 | 2.180 | 2.638 | 3.109 | 8.57 ** | 0.28 ns | 3.71 ns |
| Shoot dry weight (g) | 3.570 | 3.863 | 1.920 | 2.113 | 118.18 ** | 2.41 ns | 0.10 ns |
| Root dry weight (g) | 0.643 | 0.572 | 0.311 | 0.377 | 91.90 | 3.38 | 5.96 * |
| Root-to-shoot ratio | 0.170 | 0.156 | 0.177 | 0.180 | 0.59 ns | 0.05 ns | 0.79 ns |
| Total biomass (g) | 4.304 | 4.526 | 2.315 | 2.547 | 114.81 *** | 1.50 ns | 0.00 ns |
| Total root length (cm) | 3045.1 | 3067.3 | 1518.3 | 1936.5 | 74.58 *** | 3.28 ns | 2.25 ns |
| Specific total root length (cm) | 46.6 | 54.7 | 61.8 | 59.6 | 3.25 ns | 0.28 ns | 0.85 ns |
| Surface area ($cm^2$) | 496.9 | 510.2 | 247.0 | 322.3 | 46.55 *** | 1.91 ns | 0.93 ns |
| Average diameter (cm) | 0.053 | 0.054 | 0.052 | 0.054 | 0.00 ns | 0.12 ns | 0.02 ns |
| Total root volume | 6.593 | 6.974 | 3.220 | 4.380 | 30.06 *** | 2.66 ns | 1.34 ns |
| Adventitious root number | 22.9 | 22.0 | 26.3 | 19.5 | 0.06 ns | 5.12 * | 2.54 ns |
| Adventitious root dry weight (g) | 0.091 | 0.091 | 0.085 | 0.057 | 6.94 | 2.65 | 4.62 * |
| Adventitious root (%) | 12.8 | 13.9 | 20.8 | 12.6 | 9.34 | 11.64 | 19.20 *** |
| Adventitious root total length (cm) | 1232.6 | 1115.1 | 905.9 | 755.5 | 15.35 ** | 2.34 ns | 0.04 ns |
| Specific adventitious total length (cm) | 111.0 | 111.9 | 146.7 | 138.1 | 4.99 * | 0.06 ns | 0.01 ns |
| Adventitious root surface area ($cm^2$) | 226.8 | 202.5 | 180.4 | 144.6 | 11.31 ** | 3.75 ns | 0.14 ns |
| Adventious root average diameter (cm) | 0.060 | 0.058 | 0.066 | 0.062 | 3.36 ns | 1.03 ns | 0.12 ns |
| Adventitious root volume | 3.409 | 2.967 | 2.920 | 2.241 | 4.06 ns | 3.45 ns | 0.16 ns |

Each value in the table is the mean of eight replicates.

P: Phosphorus, G: Genotype

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.2. The effect on various developmental responses in both 'Pearson' and *Nr* tomato genotype grown in solid medium with low phosphorus (1 μM) and high phosphorus (100 μM) for 6 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-Pearson | High P-*Nr* | Low P-Pearson | Low P-*Nr* | P | G | P*G |
| Plant height (cm) | 68.9 | 69.1 | 57.9 | 57.8 | 50.23 *** | 0.00 ns | 0.01 ns |
| Branch number | 5.3 | 6.0 | 1.5 | 2.0 | 47.40 *** | 1.11 ns | 0.53 ns |
| Stem diameter (cm) | 0.79 | 0.75 | 0.70 | 0.68 | 21.86 *** | 3.51 ns | 0.18 ns |
| Leaf area ($cm^2$) | 1577.2 | 1627.0 | 1256.1 | 1238.6 | 9.94 ** | 0.02 ns | 0.09 ns |
| Specific leaf area ($mg/cm^2$) | 17.0 | 16.1 | 13.6 | 13.7 | 6.37 * | 0.01 ns | 0.03 ns |
| Shoot dry weight (g) | 14.598 | 16.793 | 9.394 | 10.741 | 74.43 *** | 7.37 * | 0.42 ns |
| Root dry weight (g) | 1.671 | 1.907 | 1.220 | 1.372 | 28.24 ** | 4.39 * | 0.21 ns |
| Root-to-shoot ratio | 0.114 | 0.114 | 0.131 | 0.129 | 9.41 ** | 0.11 ns | 0.03 ns |
| Total biomass (g) | 16.269 | 18.700 | 10.614 | 12.113 | 71.45 *** | 7.36 * | 0.41 ns |
| Adventitious root number | 28.3 | 23.3 | 40.5 | 29.9 | 24.33 *** | 16.68 *** | 2.16 ns |
| Adventitious root dry weight (g) | 0.143 | 0.182 | 0.235 | 0.163 | 8.42 | 1.70 | 18.92 *** |
| Adventitious root (%) | 8.1 | 8.7 | 16.2 | 10.7 | 67.12 | 11.77 | 21.58 *** |

Each value in the table is the mean of six to twelve replicates.

P: Phosphorus, G: Genotype

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.3. The effect on various developmental responses in both MD and *etr1-1* petunia genotype grown in solid medium with low phosphorus (1 μM) and high phosphorus (100 μM) for 4 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-MD | High P-*etr1-1* | Low P-MD | Low P-*etr1-1* | P | G | P*G |
| Plant height (cm) | 14.7 | 12.6 | 11.8 | 8.7 | 30.77 *** | 17.46 *** | 1.98 ns |
| Plant width (cm) | 28.2 | 26.0 | 19.9 | 16.6 | 32.08 *** | 3.03 ns | 0.11 ns |
| Branch number | 10.4 | 10.2 | 6.8 | 6.5 | 63.16 *** | 0.47 ns | 0.00 ns |
| Flower bud number | 6.4 | 6.1 | 0.5 | 0.3 | 55.85 *** | 0.33 ns | 0.03 ns |
| Leaf number | 97.9 | 90.2 | 59.5 | 52.3 | 91.61 *** | 3.54 ns | 0.00 ns |
| Leaf area ($cm^2$) | 1038.2 | 930.2 | 464.9 | 361.9 | 126.50 *** | 5.14 * | 0.03 ns |
| Specific leaf area ($mg/cm^2$) | 4.86 | 4.49 | 4.42 | 4.49 | 1.07 ns | 0.02 ns | 1.04 ns |
| Shoot dry weight (g) | 5.08 | 4.29 | 2.22 | 1.68 | 100.21 *** | 79.00 * | 0.02 ns |
| Root dry weight (g) | 3.36 | 2.09 | 1.49 | 1.18 | 46.43 *** | 6.29 * | 0.45 ns |
| Root-to-shoot ratio | 0.46 | 0.44 | 0.59 | 0.63 | 19.20 *** | 0.23 ns | 0.74 ns |
| Total biomass (g) | 6.94 | 6.38 | 3.72 | 2.85 | 88.97 *** | 6.78 * | 0.13 ns |
| Total root length (cm) | 71035 | 53892 | 51064 | 47131 | 3.43 ns | 2.13 ns | 0.84 ns |
| Average diameter (cm) | 0.055 | 0.060 | 0.049 | 0.044 | 11.21 ** | 0.02 ns | 2.51 ns |
| Number of internode | 5.3 | 5.5 | 5.2 | 4.8 | 6.92 | 0.00 | 0.02 * |
| Length of internode | 2.33 | 2.24 | 2.07 | 2.01 | 7.16 * | 0.69 ns | 0.02 ns |

Each value in the table is the mean of six to twelve replicates.

Two blocks were pooled for statistical analysis. Block effects were not shown.

P: Phosphorus, G: Genotype

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.4. The effect on various developmental responses in both MD and *etr1-1* petunia genotype grown in solid medium with low phosphorus (1 μM) and high phosphorus (100 μM) for 7 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-MD | High P-*etr1-1* | Low P-MD | Low P-*etr1-1* | P | G | P*G |
| Plant height (cm) | 41.4 | 36.7 | 27.3 | 19.8 | 87.16 *** | 13.24 ** | 0.12 ns |
| Plant width (cm) | 55.9 | 45.0 | 39.8 | 34.8 | 51.93 *** | 19.11 *** | 2.62 ns |
| Branch number | 15.0 | 16.8 | 10.2 | 11.3 | 71.02 *** | 5.26 * | 0.32 ns |
| Flower and bud number | 50.8 | 44.5 | 14.3 | 9.0 | 135.40 *** | 3.50 ns | 0.03 ns |
| Leaf number | 112.5 | 124.7 | 87.8 | 77.9 | 44.87 *** | 0.90 ns | 1.61 ns |
| Leaf area ($cm^2$) | 1104.5 | 1241.2 | 681.7 | 564.2 | 40.84 *** | 0.01 ns | 2.18 ns |
| Specific leaf area ($mg/cm^2$) | 23.29 | 17.09 | 9.04 | 8.95 | 40.1 ns | 2.95 ns | 2.72 ns |
| Shoot dry weight (g) | 21.4 | 19.4 | 7.7 | 6.3 | 188.52 *** | 2.98 ns | 0.05 ns |
| Root dry weight (g) | 7.1 | 6.2 | 4.0 | 3.3 | 50.73 *** | 3.92 ns | 0.04 ns |
| Root-to-shoot ratio | 0.356 | 0.330 | 0.490 | 0.507 | 14.61 *** | 1.08 ns | 0.47 ns |
| Total biomass (g) | 28.5 | 25.6 | 11.7 | 9.7 | 164.26 *** | 3.68 ns | 0.06 ns |
| Total root length (cm) | 84521 | 83944 | 66752 | 52888 | 5.91 * | 0.52 ns | 0.44 ns |
| Average diameter (cm) | 0.083 | 0.064 | 0.064 | 0.063 | 2.54 ns | 2.80 ns | 2.21 ns |
| Number of internode | 6.9 | 7.0 | 6.8 | 6.3 | 0.48 ns | 0.00 ns | 1.90 ns |
| Length of internode | 4.51 | 4.41 | 3.78 | 2.94 | 30.59 *** | 5.54 * | 3.52 ns |

Each value in the table is the mean of six to twelve replicates.

Two blocks were pooled for statistical analysis. Block effects were not shown.

P: Phosphorus, G: Genotype

*$0.05 > P = 0.01$; ** $0.01 > P = 0.001$; *** $P < 0.001$; ns, nonsignificant at the 0.05 level.

Table 2.5. The F-values from repeated measures ANOVA of Fv/Fm, CER and ETR in tomato and petunia plants grown in solid medium with low phosphorus (1 μM) and high phosphorus (100 μM) over the 7weeks of measurements after transplanting.

| Species | Source of Variance | *F* value | | |
|---|---|---|---|---|
| | | Fv/Fm | CER | ETR |
| Tomato | P level | 8.8 ** | 13.9 ** | 8.7 ** |
| | Genotype | 1.0 ns | 0.3 ns | 1.4 ns |
| | P level*Genotype | 2.6 ns | 0.2 ns | 3.1 ns |
| | Time | 52.5 *** | 25.6 *** | 52.0 *** |
| | Time*P level | 1.6 ns | 0.6 ns | 1.6 ns |
| | Time*Genotype | 0.1 ns | 0.2 ns | 0.2 ns |
| | Time*P level *Genotype | 0.5 ns | 0.2 ns | 0.3 ns |
| Petunia | P level | 174.5 *** | 179.1 *** | 81.1 *** |
| | Genotype | 34.5 *** | 31.4 *** | 21.1 *** |
| | P level*Genotype | 3.1 ns | 2.3 ns | 1.7 ns |
| | Time | 1501.8 | 1464.4 | 651.4 |
| | Time*P level | 164.4 *** | 168.3 *** | 45.7 *** |
| | Time*Genotype | 33.4 *** | 30.4 *** | 12.8 *** |
| | Time*P level *Genotype | 2.9 ** | 2.2 * | 1.0 ns |

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

CER: $CO_2$ Exchange Rate

ETR: Electron Transport Rate

Table 2.6. Changes in plant volume ($cm^3$) as affected by low phosphorus and ethylene insensitivity in tomato or petunia grown in solid medium for 6 or 7 weeks after transplanting.

| P level | Genotype | Weeks after transplanting | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tomato | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| High P | wild | 432 a | 1948 bc | 13038 a | 41625 a | 77214 a | 139236 a | 171105 a |
| | *Nr* | 461 a | 2457 a | 13743 a | 47035 a | 83809 a | 149253 a | 176169 a |
| Low P | wild | 439 a | 1619 c | 7705 b | 30339 b | 55593 b | 107816 b | 134006 b |
| | *Nr* | 453 a | 2051 ab | 8761 b | 30407 b | 56141 b | 105949 b | 133306 b |
| Petunia | | 0 | 1 | 2 | 3 | 4 | 5 | 7 |
| High P | MD | 106 a | 864 a | 2404 a | 5704 a | 10830 a | 27203 a | 69705 a |
| | *etr1-1* | 118 a | 762 a | 2042 a | 4503 ab | 9608 a | 20810 ab | 59015 ab |
| Low P | MD | 113 a | 720 a | 1410 b | 3437 b | 6296 b | 16640 b | 43708 bc |
| | *etr1-1* | 119 a | 649 a | 1256 b | 2337 c | 4007 b | 8679 c | 25802 c |

Table 2.7. The effect of phosphorus availability on various developmental responses in 'Pearson' and *Nr* tomatoes grown in hydroponic culture with low phosphorus (1 μM) and high phosphorus (100 μM) and harvested at 3 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-Pearson | High P-*Nr* | Low P-Pearson | Low P-*Nr* | P | G | P*G |
| Plant height (cm) | 32.5 | 33.8 | 17.0 | 18.0 | 498.65 *** | 2.83 ns | 0.19 ns |
| Branch number | 4.5 | 5.2 | 1.0 | 1.0 | 123.02 *** | 0.93 ns | 0.93 ns |
| Leaf number | 9.7 | 10.2 | 7.0 | 7.5 | 85.07 *** | 3.10 ns | 0.06 ns |
| Shoot dry weight (g) | 4.692 | 5.120 | 0.946 | 1.124 | 143.53 *** | 0.88 ns | 0.15 ns |
| Root dry weight (g) | 0.677 | 0.633 | 0.235 | 0.242 | 34.02 *** | 0.07 ns | 0.13 ns |
| Root-to-shoot ratio | 0.147 | 0.124 | 0.249 | 0.220 | 20.94 *** | 1.44 ns | 0.02 ns |
| Total biomass (g) | 5.369 | 5.753 | 1.181 | 1.365 | 258.07 *** | 0.84 ns | 0.12 ns |
| Total root length (cm) | 10815.7 | 9645.9 | 4856.8 | 5209.5 | 83.35 *** | 0.00 ns | 1.41 ns |
| Specific total root length (cm) | 167.2 | 159.0 | 216.8 | 223.6 | 17.39 *** | 0.00 ns | 0.30 ns |
| Surface area ($cm^2$) | 1588.0 | 1454.2 | 736.2 | 786.2 | 47.72 *** | 0.00 ns | 0.64 ns |
| Average diameter (cm) | 0.213 | 0.211 | 0.180 | 0.179 | 4.71 * | 0.00 ns | 0.11 ns |
| Total root volume | 18.7 | 17.6 | 8.9 | 9.5 | 27.57 *** | 0.02 ns | 0.28 ns |
| Adventitious root number | 26.8 | 15.7 | 19.0 | 12.3 | 5.50 * | 7.38 * | 1.03 ns |
| Adventitious root dry weight (g) | 0.088 | 0.062 | 0.079 | 0.044 | 0.16 ns | 0.02 ** | 0.43 ns |
| Adventitious root (%) | 12.3 | 9.6 | 25.5 | 19.3 | 19.93 *** | 2.72 ns | 0.23 ns |
| Adventitious root total length (cm) | 956.3 | 697.5 | 1164.7 | 1070.8 | 3.21 ns | 1.18 ns | 0.26 ns |
| Specific adventitious total length (cm) | 111.5 | 113.9 | 149.4 | 161.7 | 25.48 *** | 0.61 ns | 0.12 ns |
| Adventitious root surface area ($cm^2$) | 157.7 | 117.5 | 198.5 | 169.6 | 2.87 ns | 1.59 ns | 0.04 ns |
| Adventious root average diameter (cm) | 0.053 | 0.054 | 0.054 | 0.050 | 0.76 ns | 0.94 ns | 3.03 ns |
| Adventitious root volume | 2.087 | 1.582 | 2.724 | 2.154 | 0.87 ns | 2.17 ns | 0.08 ns |

Each value in the table is the mean of six replicates.

P: Phosphorus, G: Genotype

*$0.05 > P = 0.01$; ** $0.01 > P = 0.001$; *** $P < 0.001$; ns, nonsignificant at the 0.05 level.

Table 2.8. The effect on various developmental responses in both MD and *etr1-1* petunia genotype grown in hydroponic culture with low phosphorus (1 μM) and high phosphorus (100 μM) for 3 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-MD | High P-*etr1-1* | Low P-MD | Low P-*etr1-1* | P | G | P*G |
| Shoot dry weight (g) | 4.36 | 2.56 | 0.89 | 0.65 | 210.40 *** | 17.30 *** | 2.43 ns |
| Root dry weight (g) | 1.36 | 0.76 | 0.41 | 0.38 | 61.99 *** | 8.08 * | 3.74 ns |
| Root-to-shoot ratio | 0.31 | 0.30 | 0.46 | 0.58 | 47.38 *** | 0.08 ns | 0.35 ns |
| Total biomass (g) | 5.723 | 3.319 | 1.297 | 1.027 | 167.13 *** | 14.74 ** | 3.42 ns |
| Leaf number | 101.8 | 80.5 | 46.0 | 42.8 | 105.53 *** | 7.25 * | 3.98 ns |

Each value in the table is the mean of six to twelve replicates.

Two blocks were pooled for statistical analysis. Block effects were not shown.

P: Phosphorus, G: Genotype

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.9. The effect on various developmental responses in both MD and *etr1-1* petunia genotype grown in hydroponic culture with low P (1 μM) and high P (100 μM) for 6 weeks after transplanting.

| Variables | Treatments | | | | *F* value | | |
|---|---|---|---|---|---|---|---|
| | High P-MD | High P-*etr1-1* | Low P-MD | Low P-*etr1-1* | P | G | P*G |
| Shoot dry weight (g) | 25.2 | 20.8 | 5.2 | 4.4 | 601.30 *** | 9.38 ** | 2.25 ns |
| Root dry weight (g) | 5.623 | 3.737 | 1.764 | 1.451 | 91.09 *** | 6.62 * | 0.57 ns |
| Root-to-shoot ratio | 0.22 | 0.18 | 0.34 | 0.34 | 42.54 *** | 1.17 ns | 0.86 ns |
| Total biomass (g) | 30.825 | 24.492 | 6.935 | 5.893 | 439.46 *** | 9.55 ** | 1.89 ns |

Each value in the table is the mean of six to twelve replicates.

Two blocks were pooled for statistical analysis. Block effects were not shown.

P: Phosphorus, G: Genotype

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.10. Allometric coefficients (K) of root: shoot and adventitious root: root of 'Pearson' and *Nr* genotypes grown under low and high phosphorus. Each value is derived from paired measurements of root DW and shoot DW, or adventitious root DW and root DW (Hunt, 1990) measured at 3 and 6 weeks after transplanting.

| P level | Root:shoot | | Adventitious root:root | |
|---|---|---|---|---|
| | *K* | $r^2$ | *K* | $r^2$ |
| High P-Pearson | 0.87 a | 0.86 | 0.57 b | 0.59 |
| High P-*Nr* | 0.81 a | 0.90 | 0.60 b | 0.84 |
| Low P-Pearson | 0.84 a | 0.97 | 0.91 a | 0.91 |
| Low P-*Nr* | 0.81 a | 0.94 | 0.64 b | 0.82 |

| | *F* value | |
|---|---|---|
| P level | 0.28 ns | 9.27 ** |
| Genotype | 1.08 ns | 4.29 * |
| P level * Genotype | 0.01 ns | 3.24 ns |

*0.05 > *P* =0.01; ** 0.01> *P* =0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.11. Degree of growth depression in *Nr* tomato and *etr1-1* petunia by low phosphorus in solid medium and hydroponic culture. Each value was obtained by taking the percentage of the given value of wild-type with high phosphorus at 3 weeks for tomato and 4 weeks for petunia.

| Species | Growth medium | Phosphorus levels | Genotypes | Shoot dry weight | Root dry weight | Total Biomass | Root/Shoot Ratio | Proportion of total root dry weight in adventitious roots |
|---|---|---|---|---|---|---|---|---|
| Tomato | Solid media | High P | Pearson | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | | | *Nr* | 108.2 | 89.0 | 105.2 | 91.5 | 108.1 |
| | | Low P | Pearson | 53.8 | 48.4 | 53.8 | 104.3 | 162.0 |
| | | | *Nr* | 59.2 | 58.7 | 59.2 | 105.8 | 97.9 |
| | Hydroponics | High P | Pearson | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | | | *Nr* | 109.1 | 93.6 | 107.2 | 84.2 | 78.0 |
| | | Low P | Pearson | 20.2 | 34.7 | 22.0 | 169.3 | 207.6 |
| | | | *Nr* | 24.0 | 35.7 | 25.4 | 149.6 | 186.0 |
| Petunia | Solid media | High P | MD | 100.0 | 100.0 | 100.0 | 100.0 | n/a |
| | | | *etr* | 84.4 | 62.2 | 91.9 | 95.7 | n/a |
| | | Low P | MD | 43.7 | 44.3 | 53.6 | 128.3 | n/a |
| | | | *etr* | 33.1 | 35.1 | 41.1 | 137.0 | n/a |
| | Hydroponics | High P | MD | 100.0 | 100.0 | 100.0 | 100.0 | n/a |
| | | | *etr* | 58.7 | 55.9 | 58.0 | 96.4 | n/a |
| | | Low P | MD | 20.4 | 30.1 | 22.7 | 147.8 | n/a |
| | | | *etr* | 14.9 | 27.9 | 17.9 | 183.9 | n/a |

Table 2.12. Effects of growth media (solid medium and hydroponics) on growth responses of 3 week old 'Pearson' and *Nr* tomatoes to low phosphorus.

| Variables | *F value* | | | | | | |
|---|---|---|---|---|---|---|---|
| | GM | P | G | GM*P | GM*G | P*G | GM*P*G |
| Height (cm) | 42.00 *** | 340.54 *** | 13.71 ** | 44.76 *** | 3.35 ns | 0.02 ns | 0.19 ns |
| Shoot dry weight (g) | 5.51 * | 425.39 *** | 3.42 ns | 60.71 *** | 0.02 ns | 0.03 ns | 0.04 ns |
| Root dry weight (g) | 17.54 *** | 187.40 *** | 0.56 ns | 16.90 *** | 0.49 ns | 3.44 ns | 0.00 ns |
| Root-to-shoot ratio | 0.43 ns | 23.30 *** | 1.28 ns | 12.31 ** | 0.46 ns | 2.06 ns | 1.36 ns |
| Total biomass (g) | 12.31 ** | 456.15 *** | 2.42 ns | 61.77 *** | 0.01 ns | 0.24 ns | 0.01 ns |
| Adventitious root number | 22.42 *** | 3.46 ns | 11.87 ** | 1.15 ns | 2.03 ns | 0.12 ns | 1.29 ns |
| Adventitious root dry weight (g) | 6.06 * | 14.69 *** | 13.02 *** | 0.62 ns | 3.57 ns | 2.89 ns | 0.00 ns |
| Adventitious root (%) | 0.41 ns | 32.45 *** | 16.44 *** | 10.46 ** | 1.66 ns | 10.46 * | 0.40 ns |
| Adventitious root total length (cm) | 0.12 ns | 0.09 ns | 3.24 ns | 13.54 ** | 0.06 ns | 0.15 ns | 0.33 ns |
| Total root length (cm) | 439.38 *** | 136.23 *** | 1.83 ns | 0.21 ns | 2.26 ns | 3.56 ns | 0.18 ns |

GM: Growth medium, P: Phosphorus, G: Genotype

*0.05 > *P* =0.01; ** 0.01> *P* =0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 2.13. Effects of growth media (solid medium and hydroponics) on growth responses of 3 or 4 week old MD and *etr1-1* petunias to low phosphorus.

| Variables | *F value* | | | | | | |
|---|---|---|---|---|---|---|---|
| | GM | P | G | GM*P | GM*G | P*G | GM*P*G |
| Shoot dry weight (g) | 13.57 *** | 90.65 *** | 6.61 ** | 1.33 ns | 0.34 ns | 0.54 ns | 0.43 ns |
| Root dry weight (g) | 15.51 *** | 25.85 *** | 3.30 ns | 0.29 ns | 0.01 ns | 0.71 ns | 0.09 ns |
| Root-to-shoot ratio | 4.87 * | 18.71 *** | 0.43 ns | 0.36 ns | 0.03 ns | 0.37 ns | 0.24 ns |
| Total biomass (g) | 10.48 ** | 43.91 *** | 2.88 ns | 1.16 ns | 0.45 ns | 0.17 ns | 0.73 ns |

GM: Growth medium, P: Phosphorus, G: Genotype

*0.05 > *P* =0.01; ** 0.01> *P* =0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

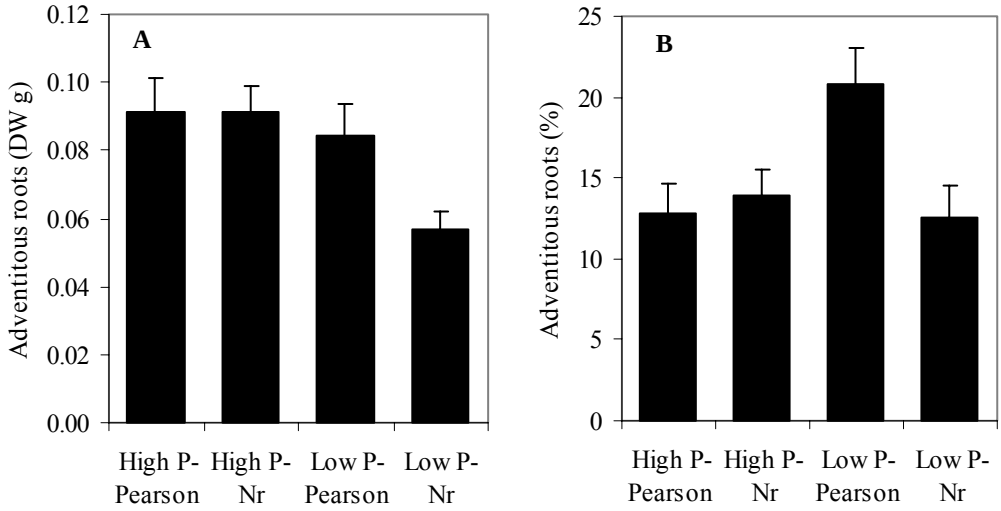


Figure 2.1. (A) The effect of low phosphorus and *Nr* on dry weight of adventitious roots of tomato plants grown in solid medium at 3 weeks after transplanting. (B) The effect of low phosphorus and *Nr* on the percentage of adventitious root dry weight (g) out of total root dry weight (g) of tomato plants at 3 weeks after transplanting. Error bars represent ± SE; n=14.

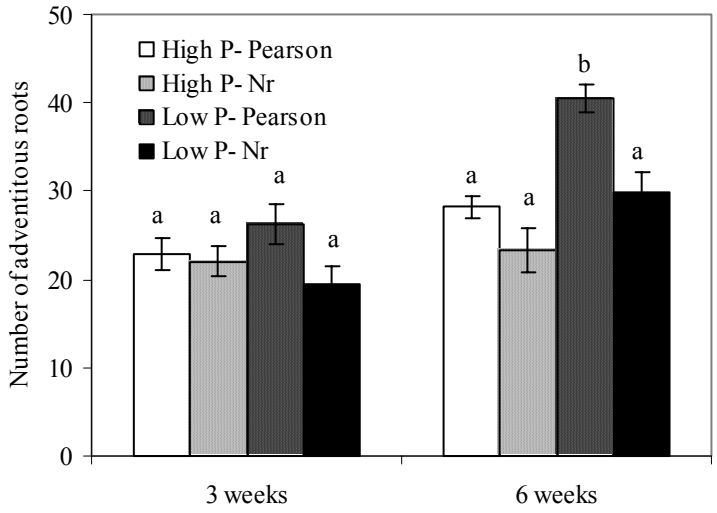


Figure 2.2. The effect of low phosphorus and *Nr* on the number of adventitious roots of tomato plants grown in solid medium at 3 weeks after transplanting. Error bars represent ± SE; n=14.

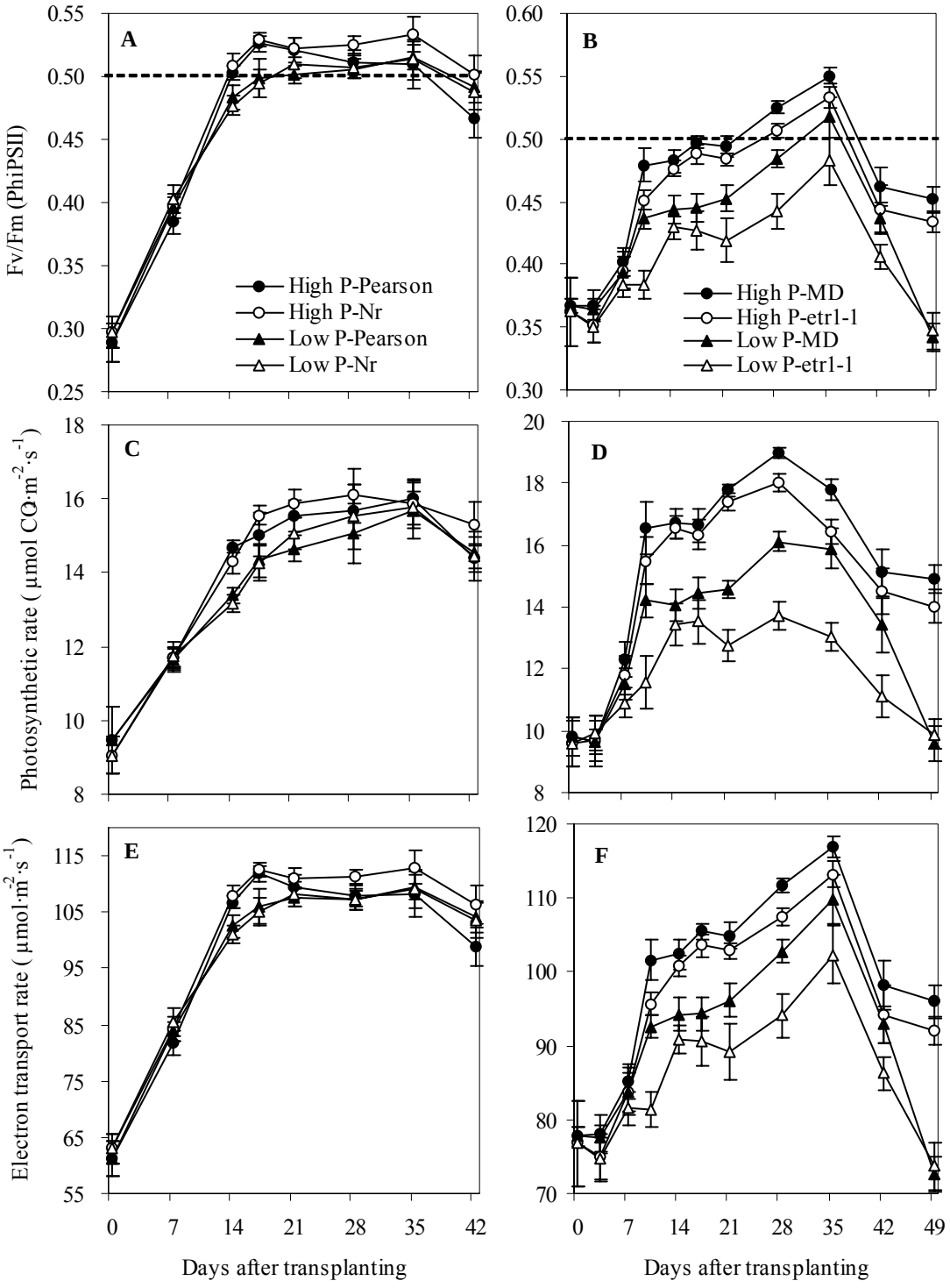


Figure 2.3. The effect of low P (1µM) on (A) chlorophyll fluorescence (Fv/Fm), (C) $CO_2$ exchange rate and (E) electron transport rate of 'Pearson' and *Nr* tomato plants during first 6 weeks after transplant. n=8. The effect of low P on (B) chlorophyll fluorescence (Fv/Fm), (D) $CO_2$ exchange rate and (F) electron transport rate of MD and *etr1-1* petunia during the first 7 weeks after transplanting. n=8. Error bars represent ± SE.

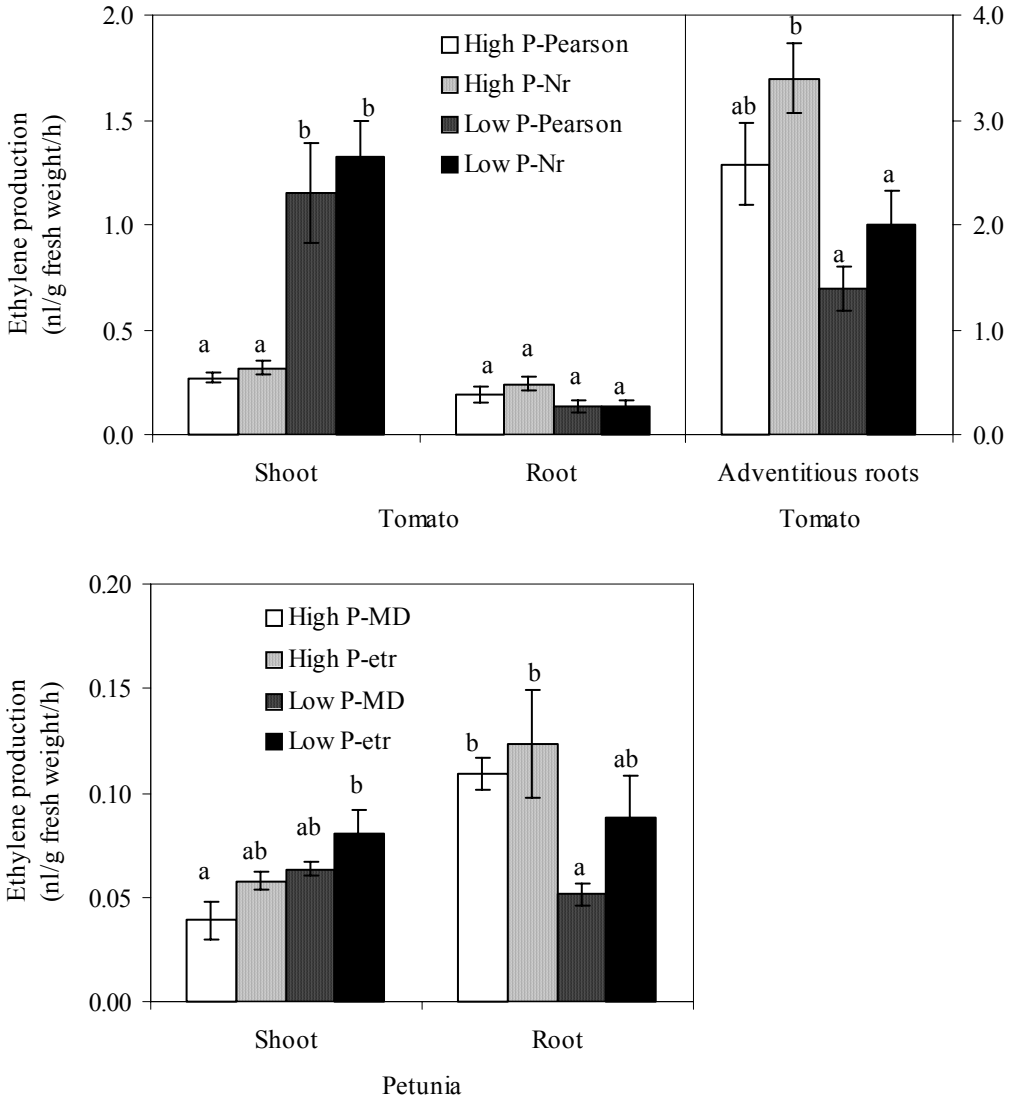


Figure 2.4. Ethylene production of shoot, root, and adventitious roots in tomato and petunia grown under low phosphorus at 3 (tomato) and 4 weeks (petunia) after transplanting. Error bars represent ± SE; n=6 or 8. Note variation in scale of y-axis.

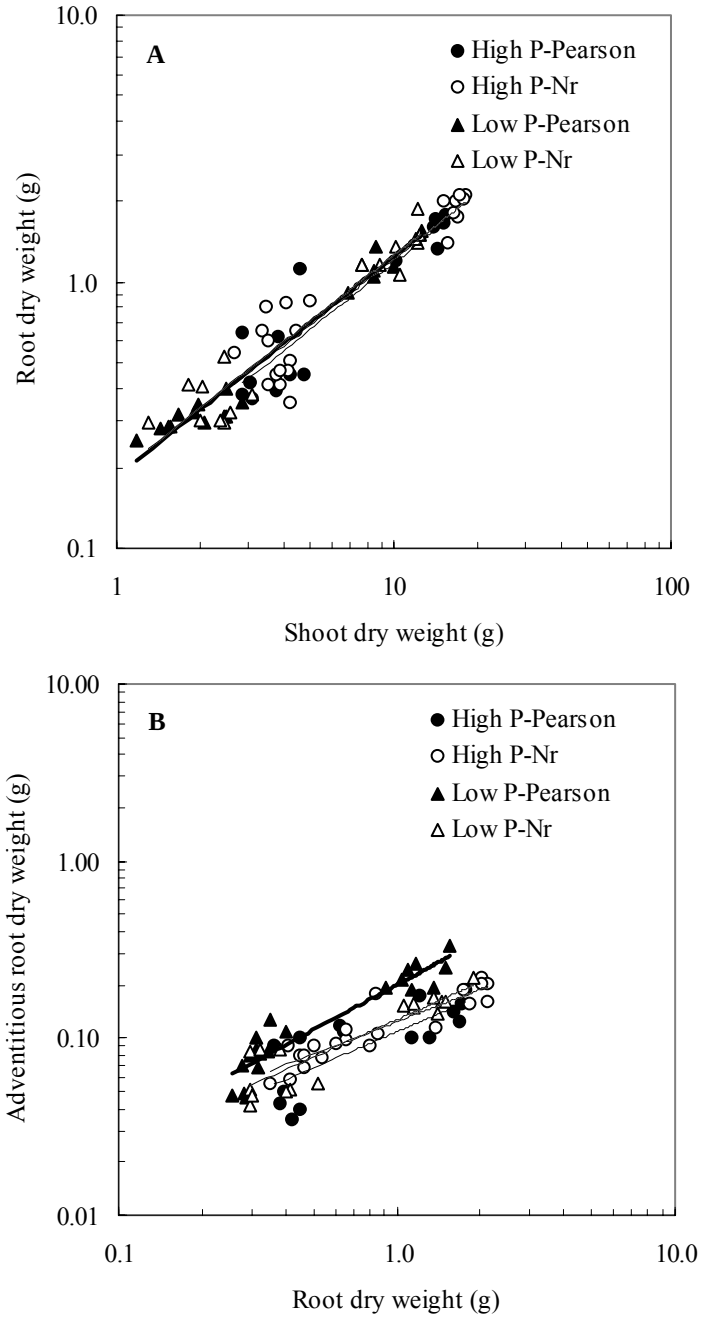


Figure 2.5. Allometric relationships between (A) the root dry weight and shoot dry weight and (B) the adventitious root dry weight and root dry weight of 'Pearson' and *Nr* genotypes grown under low and high phosphorus measured at 3 and 6 weeks after transplanting. n≥8.

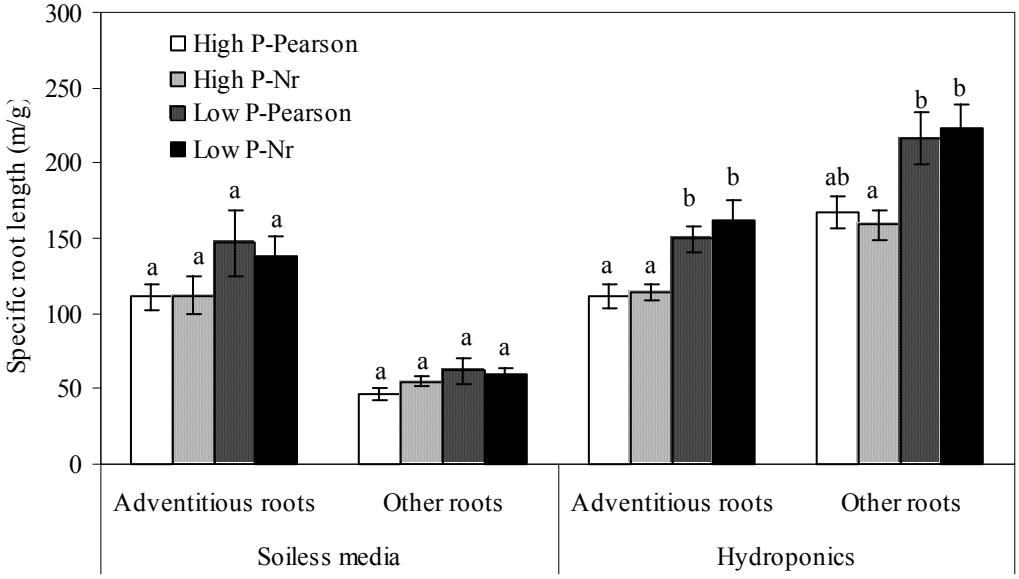


Figure 2.6. The effect of low phosphorus and hydroponic culture on specific root length of adventitious root and other roots in tomato plants at 3 weeks after transplanting. Error bars represent ± SE; n=6 or 8.

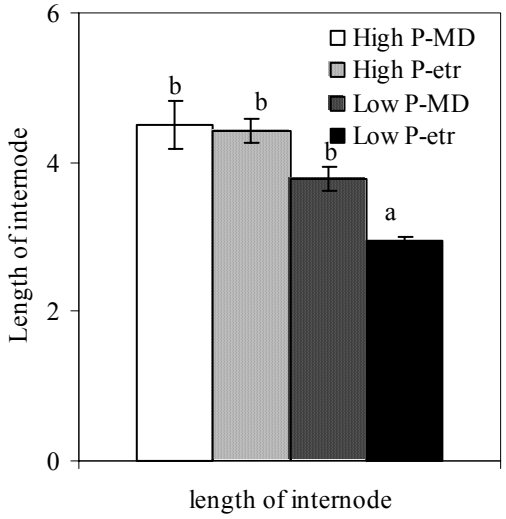


Figure 2.7. The effect of low phosphorus and Nr on the number of adventitious roots of tomato plants grown in solid medium at 7 weeks after transplanting. Error bars represent ± SE; n=14.

# Chapter 3

## Disease development in ethylene insensitive *etr1-1* petunia infected by *Thielaviopsis basicola* under low phosphorus stress


## ABSTRACT

Low phosphorus dramatically enhanced disease symptom development, as measured by percent necrotic leaves, in both wild-type and *etr1-1* petunias during a 7 week period after transplanting. Ethylene insensitive *etr1-1* petunia developed earlier and more severe disease symptoms than the wild-type during the first 4 weeks after transplanting, but showed less severe disease at 7 weeks as compared to the wild-type, indicating that ethylene plays a role in symptom development. Disease symptom development was positively correlated with the number of colonies that grew from root segments plated on a *Thielaviopsis* selective medium (TB-CEN) in both wild-type and *etr1-1* petunia. The *etr1-1* petunias displayed more severe disease development symptoms compared to wild-type when the roots were equally infected by *T. basicola*. Inoculation methods and environmental conditions considerably influenced disease severity.

## INTRODUCTION

*Thielaviopsis basicola* is a soil-borne, plant-pathogenic fungus devastating many bedding plants and floricultural crops including pansy, poinsettia, *Petunia*, vinca, *Cyclamen*, snapdragon, *Impatiens*, *Verbena*, *Phlox*, *Begonia*, and *Nicotiana* (Daughtrey and Simone, 1995; Pirone, 1978). The survival structures of this fungus, when they are present in high numbers, produce black regions on plant roots, hence the common name "black root rot". The plants with black root rot show stunted shoot growth, and yellow or necrotic areas at the tips of older leaves. Black root rot is favored by cool soil temperatures (17-23 ºC), high soil moisture, and neutral or alkaline soil pH (Agrios, 1997; Lucas, 1975), all of which are common in horticultural production. When conditions are unfavorable for infection, *T. basicola* forms survival structures that may be found in used soil or plant debris. Without proper sanitation, these serve as a source of *T. basicola* in the production area.

Despite the significance of black root rot disease, little is known about how the disease is influenced by production practices, especially nutrition. Results from previous research indicate that phosphorus may play a role in severity of root rot diseases. The incidence of root rot disease caused by *Rhizoctonia solani*, *Fusarium culmorum* and *Pythium spp*. was diminished by seed-placed phosphorus fertilizer in wheat and barley (Cook, 2001; Lafond et al., 1996). Phosphorus fertilization reduced leaf rust severity (*Puccinia triticina*) and increased yield in wheat compared with an unfertilized control (Sweeney et al., 2000). Conversely, the application of phosphorus increased root rot severity caused by *Cochliobolus sativus* and *Aphanomyces euteiches* in wheat and pea

roots, respectively (Bodker et al., 1998; Goos et al., 1994), and did not alter the percentage of root necrosis and the number of propagules of *F. oxysporum* in tomato plants (Caron et al., 1986).

It is unknown how phosphorus nutrition influences development of black root rot disease. Ethylene could be involved, since low phosphorus can increase ethylene production (Borch et al., 1999). Pathogen infection often results in increased production of ethylene, which could be an important signal for plant defense mechanisms against pathogen invasion (Reymond and Farmer, 1998). However, it is unclear whether ethylene does more to promote disease susceptibility or disease resistance.

Ethylene perception seems to be required for plant resistance to bacterial and fungal pathogens. Transgenic ethylene-insensitive tobacco plants (Tetr) showed stem browning when grown in non-autoclaved potting soil from which *Thielaviopsis basicola*, *Fusarium spp.*, *Pythium spp*., and *Rhizopus stolonifer* were isolated (Knoester et al., 1998). Ethylene-insensitive soybean mutants were more susceptible to *Septoria glycines* and *Rhizoctonia solani* (Hoffman et al., 1999). Similarly, *Arabidopsis* mutant *ein2-1* demonstrated increased susceptibility to *B. cinerea* (Thomma et al., 1999). In contrast, *Nr* mutant tomato with reduced ethylene sensitivity displayed fewer disease symptoms after inoculation with bacterial (*Xanthomonas campestris* and *Pseudomonas syringae*), and fungal (*Fusarium oxysporum*) pathogens (Lund et al., 1998). Reduced disease symptom development was reported in ethylene-insensitive *Arabidopsis* mutant *ein2-1* after infection with virulent bacterial strains of *Pseudomonas syringae* and *Xanthomonas campestris* (Bent et al., 1992).

In preliminary experiments, we observed chlorotic and necrotic leaves developing from the lower stem of petunia infected by *Thielaviopsis basicola* (Figure 3.1). The infected plants did not show either stunted shoot growth or blackened roots, which are characteristic of severe infections (Daughtrey and Simone, 1995), implying that they were undergoing mild infections. The absence of typical root symptoms prompted us to question whether the severity of shoot disease symptoms is directly related to the extent of root infection. Interestingly, the *etr1-1* petunia showed enhanced disease development symptoms especially under low phosphorus, but still lacked visible root symptoms. This research was conducted to elucidate how disease development symptoms are influenced by the interaction of phosphorus nutrition and ethylene sensitivity, and whether a correlation exists between the degree of disease symptom development in the shoot and the fungal population in infected roots.

## MATERIALS AND METHODS

### Plant materials

Inbred cv. Mitchell Diploid (wild-type) and transgenic (Wilkinson et al., 1997) *etr1-1* petunia (*Petunia* x*hybrida*) plants were obtained from Dr. David G. Clark at the University of Florida. The seeds were soaked in 100 $\mu l \cdot l^{-1}$ Gibberellic Acid ($GA_3$) overnight and washed thoroughly with distilled water. The seeds of both genotypes were germinated in a 16 × 8 plug tray filled with Aggregate Plus Media – Sunshine Mix-4 containing starter fertilizer (SUN GRO Horticulture, Bellevue, Washington) and placed in a greenhouse under intermittent misting with tap water. Three to four week-old

seedlings were transplanted into 10 cm pots at the time the leaves began to contact neighboring plants, i.e. plant diameter was approximately 4 cm. The potting medium was autoclaved twice prior to the experiment. The plants were grown in a greenhouse at University Park, PA, USA (40.79º N, 77.86º W). Day and night temperatures in the greenhouse during the experiment period were 28 ºC ± 3 and 22 ºC ± 2 for the root culture experiment, and 23 ºC ± 0.1 and 19 ºC ± 0.1 for disease severity experiment.

**Nutrient solution**

Plants were grown in solid medium (40:40:20 v/v/v=vermiculite:perlite:sand) containing 1.5 % (w/v) solid-phase phosphorus buffered alumina-P (Lynch et al., 1990) providing two desorption concentrations, low P (1 µM) and high P (100 µM), allowing a constant but a regulated supply of phosphorus. Pots were automatically irrigated once a day while the plants were small, and twice a day as the plants grew older with nutrient solution of pH 6.2-6.4 containing (in µM) 1500 $KNO_3$, 1200 $Ca(NO_3)_2$, 400 $NH_4NO_3$, 25 $MgCl_2$, 5 Fe-EDTA, 500 $MgSO_4$, 300 $K_2SO_4$, 300 $(NH_4)_2SO_4$, 1.5 $MnSO_4$, 1.5 $ZnSO_4$, 0.5 $CuSO_4$, 0.15 $(NH_4)_6Mo_7O_{24}$ and 0.5 $Na_2B_4O_7$. Nutrient solution was provided by a dripper installed to each pot. High and low phosphorus treatments contained 100 µM and 1 µM $KH_2PO_4$, respectively.

**Experiment 1: Inoculum preparation and root culture**

*T. basicola* was isolated from infected petunia roots and grown on potato – dextrose agar medium at 20 C. Conidia were washed from the agar plates with sterilized distilled water. The suspension was filtered through several layers of cheese cloth to

remove mycelia and was adjusted to a concentration of $10^6$ conidia per ml. Four week old petunia seedlings were fertigated with nutrient solution and inoculated with conidia at the stem base by pouring 10 ml spore suspension to establish 0, $10^3$, or $10^4$ fungal spores per g soil. After the inoculation, fertigation was withheld for one day to prevent the fungal spores from washing out of the medium, and was resumed next day. Nutrient solution was applied without leaching for a week.

At the time of harvest, the roots were carefully removed from the pot, thoroughly rinsed with running distilled water and stored in 5 ºC until processed. One hundred 1-cm root segments with lateral root attached were randomly selected and plated out on the *Thielaviopsis* selective medium (TB-CEN; *T. basicola*-carrot-etridiazol-nystatin) as described by (Specht and Griffin, 1985) in 10 cm Petri dishes. Twenty five root segments were plated per Petri dish. The Petri dishes were incubated at room temperature (20-22 ºC) for at least 10 days. Colonies grown from root segments were examined microscopically to ensure that they were *T. basicola*. The number of colonies was counted if they exhibited typical colonies of *T. basicola* characterized by radially developed gray-color. The experiment was done from June to July 2002.

**Experiment 2: Inoculum density and assessment of disease severity**

Three week old petunia seedlings in plug trays were moved to the greenhouse. Fungal suspensions were prepared as described above. The suspension was well shaken and poured into a container (31 x 23 x 10 cm) at appropriate concentrations so that when the plug trays were placed inside the container to soak the soil, there were 0, $10^3$, or $10^4$ spores per g soil. The inoculated seedlings were incubated in a greenhouse for a week to

facilitate fungal growth, and transplanted and grown as described above. Soil temperature was monitored with a soil thermometer and ranged from 18 to 20 ºC.

At the time of harvest, the numbers of yellow leaves and leaves with necrotic lesions were recorded. Disease severity was quantified on the basis of the proportion of necrotic leaves out of total leaf number since there were no apparent necrotic lesions observed in petunia roots. At the time of harvest, numbers of leaves along the main stem and branches were recorded. This experiment was conducted from November 2003 to January 2004.

**Statistical analyses**

The tests of inoculum density and assessment of disease severity were completely randomized designs consisting of 2 phosphorus levels, 2 genotypes and 3 inoculation densities. The following multiple linear regression model was fit for inoculum density and disease severity.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2$$

where Y = number of colonies, $X_1$ = necrotic leaves (%) and $X_2$ = dummy variable for genotype ($X_2$ = 0 for *etr1-1*, $X_2$ = 1 for wild-type petunia). Regression functions for each genotype were as follows.

*etr1-1* ($X_2 = 0$): $Y = b_0 + b_1X_1$

wild-type ($X_2 = 1$): $Y = (b_0 + b_2) + (b_1 + b_2)X_1$

The slopes of the relationship between two variables were used as indices of the degree of root infection (Hammer and Evensen, 1994). A partial F-test was used to test whether the two genotypes had equal slopes. Phosphorus, genotype and inoculum density

effects were tested at $P < 0.05$. Data were analyzed using Minitab (Minitab Inc.). When the data were not normally distributed, they were transformed with Box-Cox transformation, allowing data to be normally distributed. Arcsine transformation was conducted for analyzing disease development symptoms. The number of yellow or necrotic leaves was square-root transformed. Then they were analyzed with ANOVA general linear model.

## RESULTS

### Ethylene insensitive plants had more disease at 4 weeks after transplanting, but less at 7 weeks

When uninoculated plants were grown in unautoclaved potting medium, plants developed disease symptoms characteristic of infection with *T. basicola*. Low phosphorus availability significantly enhanced disease development symptoms in both wild-type and ethylene insensitive genotypes (Figure 3.2). Ethylene insensitive plants had more symptoms than wild-type plants at 4 weeks after transplanting, but less severe symptoms than wild-type at 7 weeks (Figure 3.2). However, low phosphorus continued to result in higher disease development throughout the experimental period. Disease development symptoms appeared earlier with low phosphorus in both genotypes, and earlier in *etr1-1* petunia relative to wild-type regardless of phosphorus levels (Figure 3.3). Even with the difference in disease development symptoms, plant growth of *etr1-1* was not different from wild-type in the same phosphorus levels (Table 3.1). There were significant

phosphorus effects on total leaf number, and the numbers of branches and leaves along the main stem (Table 3.1).

**Root infection and shoot disease development symptoms**

Plants were inoculated with fungal suspensions to investigate whether there was a correlation between root infection and shoot disease development symptoms. Root infection was evaluated using wild-type and *etr1-1* petunia grown in high phosphorus (Figure 3.4). Despite the application of high inoculum density, disease development symptoms in wild-type and *etr1-1* ranged only to 3.8 % (Figure 3.4), similar to the previous experiment (Figure 3.2), but with more symptom development in wild-type.

Disease symptoms were positively correlated with the number of colonies that developed from root segments plated on TB-CEN medium in both wild-type and *etr1-1* petunia (Figure 3.4). The intercepts of the estimated regression functions for the two genotypes were not different. However, the slope for *etr1-1* petunia was significantly steeper than that for wild-type at $p < 0.001$ level. ANOVA results indicated that the number of colonies was significantly affected by inoculum density at $p < 0.01$ but not by genotype, and that both genotype and inoculum density had significant effects on % of necrotic leaves at $p < 0.05$ level (Table 3.2).

**Inoculum density and disease severity**

When inoculated seedlings were incubated in a greenhouse for a week prior to transplanting, inoculum density significantly increased shoot disease development

(number of necrotic leaves) in both wild-type and *etr1-1* petunia (Figure 3.5 and Table 3.3).

The *etr1-1* genotype developed more severe disease symptoms at 4 weeks after transplanting compared to wild-type when they were inoculated with the same inoculum density (Figure 3.5 and Table 3.3). The *etr1-1* petunia developed significant disease symptoms even under high phosphorus, while wild-type petunias did not show much disease development even when they were inoculated with $10^4$ conidia (Figure 3.5). Low phosphorus significantly enhanced disease development symptoms in both genotypes, but more severely in *etr1-1* (Figure 3.5). Unlike the previous observations, increase in inoculum density in *etr1-1* significantly reduced the numbers of total leaves, leaves along the main stem, and branches irrespective of phosphorus level (Figure 3.6 and Table 3.3), indicating that plant growth of *etr1-1* petunia was dramatically retarded by the inoculation with fungal spores.

## DISCUSSION

Low phosphorus significantly enhanced symptoms of *Thielaviopsis basicola* infection in greenhouse-grown petunia plants. When seven week old seedling geraniums were grown in suboptimum, optimum, or excessive concentration of phosphorus, mortality of the inoculated plants to *Pythium ultimum* increased as the P concentration increased (Gladstone and Moorman, 1989). In our experiments, the concentration used for high phosphorus treatment actually provided optimal concentration for plant growth, while low phosphorus was extremely low (deficient), and the phosphorus levels were

constantly maintained by using buffered alumina-P (Lynch et al., 1990). Our results combined with those of Gladstone and Moorman (1989) demonstrate that greater disease severity may be associated with both deficient and excessive phosphorus nutrition. The effects of phosphorus on disease severity were inconsistent among previous reports on field-grown crops (Bodker et al., 1998; Caron et al., 1986 ; Goos et al., 1994; Sweeney et al., 2000). These contradictory results on phosphorus effects on disease severity may be partly due to different concentrations or sources of phosphorus employed in each experiment, or to different host-pathogen interactions.

Disease development also varied with inoculation schemes and environmental conditions. When we inoculated after transplanting during summer, disease development symptoms ranged to only 10% of leaves affected (Figure 3.4). However, when 3-week old seedlings were inoculated one week prior to transplanting during winter, up to 40% of leaves exhibited symptoms (Figure 3.5). The optimum temperature for development of black root rot of tobacco plants is 17 to 23 ºC, at which this weak pathogen can cause maximum disease development (Agrios, 1997). The temperature during our winter experiment (19-23 ºC) was more favorable for disease development compared to the temperature during the summer experiment (22-28 ºC). These differences could explain much of the variation in the disease severity shown in our experiments. Petunia plants infected by *T. basicola* did not show typical black necrotic lesions in roots or the distinctive impairment in shoot development when the experiment was conducted in summer. The only visual symptoms were the development of leaf chlorosis and necrosis of the lower stem. During severe infection with *T. basicola*, plant shoots typically display stunted growth, and the root system becomes black and water-soaked (Daughtrey and

Simone, 1995), and these symptoms were observed in *etr1-1* petunia infected by *T. basicola* when the experiment was conducted in winter (Table 3.4 and Figure 3.6). The lack of typical disease symptoms in some experiments could have resulted from mild infection of *T. basicola*. These mild disease symptoms seemed to be caused by a low concentration of fungal conidia, since conditions more favorable to fungal growth immediately led to stunted shoot growth.

Shoot symptoms were greater in the ethylene insensitive genotype during the first four weeks (Figure 3.2). However, disease progress slowed relative to wild-type resulting in less disease development in *etr1-1* by 7 weeks after transplanting. Plants grown at low phosphorus had greater symptom development throughout plant development in both genotypes (Figure 3.2). The lack of significant interactions between phosphorus nutrition and genotype, and the reversal of *etr1-1* effects between 4 and 7 weeks after transplanting, imply that *etr1-1* and low phosphorus have distinct effects on susceptibility to this disease, and that ethylene insensitivity makes the plant more susceptible only early in development. Reversal effect of ethylene insensitivity on disease development was demonstrated in other host-pathogen interactions. Ethylene treatment of tobacco leaves prior to inoculation with tobacco mosaic virus (TMV) caused an early cessation of lesion growth, while ethylene treatment during lesion development contributed to lesion expansion (Ross and Pritchard, 1972). The ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and ethephon (ethylene releasing compound) reduced the size of primary lesions caused by tobacco mosaic virus (TMV) when applied before or shortly after virus inoculation. However, lesion expansion was inhibited when primary infected leaves were treated with inhibitors of ACC-synthase (1-aminoethoxyvinylglycine), ACC-

oxidase (cobalt chloride) or ethylene action (silver nitrate and 2,5-norbornadiene) (Knoester et al., 2001). These results indicate that disease progression is a complex process, and ethylene has opposite effects on different processes within that progression. The effect of ethylene could be also altered by plant age. Two week old seedlings of ethylene-insensitive transgenic tobacco plants (Tetr) were more susceptible to *Fusarium* isolates compared to non-transformed plants, but at 6 weeks, Tetr plants were less susceptible compared to wild-type (Geraats et al., 2003). The ethylene-insensitive 6 week old *Nr* tomato displayed fewer disease symptoms after inoculation with *Fusarium oxysporum* (Lund et al., 1998). Therefore, we concluded that disease development is affected by ethylene to an extent that varies with plant age, and that ethylene protects young plants but it accelerates disease development symptoms in older plants.

How might ethylene insensitivity increase disease severity during early development? The increased susceptibility of *etr1-1* petunia could be caused by the inability to express pathogenesis-related (PR) genes. It was demonstrated that the basic PR gene expression was dependent on ethylene perception, and the impairment of this expression resulted in the loss of nonhost resistance against soil-borne pathogens (Knoester et al., 1998). Total peroxidase activities of *Arabidopsis* mutant and transgenic tobacco plants expressing *etr1-1* were lower than that of wild-types (Bleecker et al., 1988; Geraats et al., 2003). However, in the absence of ethylene-dependent defense responses, ethylene insensitive genotypes may exert ethylene-independent disease resistance, presumably via jasmonate (JA)- or salicylic-acid (SA)-dependent defense responses, depending on the pathogen. JA-dependent defense responses seem to be more effective against necrotrophic pathogens, whereas SA-dependent defense responses are

more effective against biotrophic pathogens (Thomma et al., 2001). Considering that *T.basicola* is a necrotroph, the disease response of *etr1-1* petunia may be dependent on JA-signaling.

Under conditions favorable for disease development, the shoots of *etr1-1* petunia treated with high inoculum density became remarkably stunted regardless of phosphorus level (Table 3.3 and Figure 3.6) with severe development of disease symptoms (Figure 3.5). When the plants are this severely infected, the previously observed slowing of disease development of *etr1-1* relative to wild-type is not likely to occur. Microscopic observation revealed that the chlamydospores of *T. basicola* and oospores of *Pythium* coexisted in the same root-rot lesion, while non-inoculated *etr1-1* grew normally without any indication of infection by other pathogens. The increased disease symptoms caused by *T. basicola* appeared to make *etr1-1* petunia more susceptible to *Pythium*, exacerbating disease severity especially under low phosphorus conditions.

Disease symptoms were positively correlated with the number of colonies that developed from root segments plated on a *Thielaviopsis* selective medium (TB-CEN) in both wild-type and *etr1-1* petunia. We expected a correlation between infection and disease symptoms, based on a significant positive correlation between incidence of root infection and disease severity in *T. basicola* infected tobacco plants (Wilkinson et al., 1995). Since no root rot lesions were visually observable in petunia, disease severity was evaluated based on the percent of necrotic leaves. The regression analysis of the relationship between disease development symptoms and the number of colonies grown out of TB-CEN medium demonstrates that *etr1-1* petunias displayed more severe disease development symptoms compared to wild-type petunia when the roots were equally

infected by *T. basicola* (Figure 3.4). Relatively low correlation ($r^2$=0.65) might have resulted from the fact that development of necrotic leaves is not a direct result of fungal invasion but secondary effect caused by impairment of root tissue function and consequent cell death (Lucas, 1975). Nevertheless, our regression analysis provides general information on how these variables are related to each other and how ethylene insensitivity enhances disease development in petunia infected by *T. basicola*. Further work is needed to clarify how ethylene insensitivity increases shoot disease symptoms in earlier stages of infection, and reduces them at later stages under mild infection with *T. basicola*.

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Table 3.1. The effect of ethylene insensitivity (*etr*) on disease development symptoms and various developmental responses in uninoculated petunias infected by *T. basicola*. Plants were grown in solid medium with low phosphorus (1 μM) or high phosphorus (100 μM) for 4 weeks after transplanting.

| Treatments | Disease development symptoms (%) | Total leaf number | Number of leaves along the main stem | Number of branches |
|---|---|---|---|---|
| High P-WT | 0.3 (0.3) | 97.9 (3.2) | 21.3 (0.2) | 10.4 (0.4) |
| High P-etr | 5.3 (0.5) | 90.2 (3.9) | 22.2 (0.3) | 10.2 (0.2) |
| Low P-WT | 9.5 (1.3) | 59.5 (5.5) | 20.7 (0.6) | 6.8 (0.5) |
| Low P-etr | 13.6 (1.6) | 52.3 (3.6) | 19.3 (0.5) | 6.5 (0.5) |
| | *F-value* | | | |
| Phosphorus | 25.97 *** | 91.61 *** | 12.75 ** | 63.2 *** |
| Genotype | 17.79 *** | 3.54 ns | 0.26 ns | 0.5 ns |
| P*G | 1.41 ns | 0.00 ns | 4.88 * | 0.0 ns |

WT: wild-type petunia

P: Phosphorus level

G: Genotype

Disease development symptoms were calculated based on the proportion of the number of total leaves as necrotic leaves

Mean (standard error) of at least six plants

*0.05 > *P*=0.01; ** 0.01> *P*=0.001; *** *P* < 0.001; ns, nonsignificant at the 0.05 level.

Table 3.2. The F-values from ANOVA of number of colonies and % necrotic leaves in wild-type and *etr1-1* petunia grown with high phosphorus (100 μM) at 3 or 4 weeks after transplanting.

| Source of variance | *F-value* | |
|---|---|---|
| | *Number of colonies* | *% Necrotic leaves* |
| Genotype | 0.40 ns | 4.95 * |
| Inoculum density | 4.50 ** | 3.74 * |
| Genotype* Inoculum density | 0.21 ns | 0.08 ns |

Disease development symptoms were calculated based on the proportion of the number of total leaves as necrotic leaves. An arcsine transformation was used on the % necrotic leaves.

*0.05 > $P$=0.01; ** 0.01> $P$=0.001; *** $P$ < 0.001; ns, nonsignificant at the 0.05 level.

Table 3.3. The effect of inoculum density on plant and disease development in wild-type and *etr1-1* petunias grown in solid medium with low phosphorus (1 µM) or high phosphorus (100 µM) for 4 weeks after transplanting. Petunia seedlings were inoculated with various concentrations of *T. basicola* fungal spores one week prior to transplanting.

| Treatments | Total leaf number | Disease development symptoms (%) | Number of leaves along the main stem | Number of branches |
|---|---|---|---|---|
| High P-WT-control | 90.0 (3.9) | 0.0 (0.0) | 23.5 (1.8) | 6.5 (0.3) |
| High P-WT-$10^3$ | 94.5 (4.6) | 0.3 (0.3) | 24.8 (0.6) | 6.5 (0.3) |
| High P-WT-$10^4$ | 90.3 (4.8) | 0.8 (0.5) | 23.3 (1.1) | 6.8 (0.3) |
| High P-etr-control | 102.3 (8.2) | 0.0 (0.0) | 21.5 (0.3) | 7.8 (0.6) |
| High P-etr-$10^3$ | 15.3 (0.5) | 23.0 (0.3) | 15.3 (0.5) | 1.0 (0.0) |
| High P-etr-$10^4$ | 14.8 (0.8) | 27.5 (0.4) | 14.8 (0.8) | 1.0 (0.0) |
| Low P-WT-control | 64.8 (1.0) | 0.0 (0.0) | 18.3 (0.3) | 6.5 (0.3) |
| Low P-WT-$10^3$ | 63.8 (1.7) | 11.0 (0.9) | 18.4 (0.2) | 6.4 (0.2) |
| Low P-WT-$10^4$ | 61.7 (1.2) | 17.0 (1.2) | 18.0 (0.3) | 6.3 (0.2) |
| Low P-etr-control | 76.5 (1.6) | 0.0 (0.0) | 18.3 (0.3) | 7.8 (0.3) |
| Low P-etr-$10^3$ | 11.0 (0.9) | 36.3 (0.4) | 11.0 (0.9) | 1.0 (0.0) |
| Low P-etr-$10^4$ | 10.8 (0.5) | 39.7 (0.5) | 10.8 (0.5) | 1.0 (0.0) |
| | | *F-value* | | |
| Phosphorus | 161.33 *** | 109.8 *** | 129.5 *** | 0.3 ns |
| Genotype | 1520.17 *** | 285.9 *** | 193.4 *** | 1995.8 *** |
| Inoculum density | 500.88 *** | 312.2 *** | 29.4 *** | 665.4 *** |
| P*G | 6.66 * | 18.8 *** | 4.6 * | 0.5 ns |
| P*ID | 0.05 ns | 27.0 *** | 0.5 ns | 0.3 ns |
| G*ID | 496.88 *** | 70.0 *** | 32.4 *** | 664.6 *** |
| P*G*ID | 0.83 ns | 5.0 ** | 0.1 ns | 0.2 ns |

WT: wild-type petunia
P: Phosphorus level
G: Genotype
ID: Inoculum density

Disease development symptoms were calculated based on the proportion of the number of total leaves as necrotic leaves
Mean (standard error) of at least four plants
*$0.05 > P = 0.01$; ** $0.01 > P = 0.001$; *** $P < 0.001$; ns, nonsignificant at the 0.05 level.


Figure 3.1. Disease symptoms developing from the lower stem of petunia infected by *Thielaviopsis basicola.*

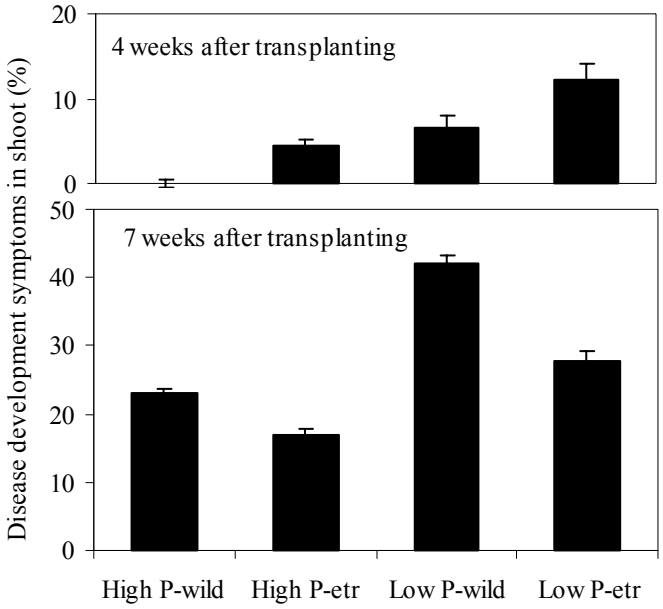


Figure 3.2. The effect of *T. basicola* on shoot disease development symptoms as indicated by percentage of necrotic leaves out of total leaves in wild-type and *etr1-1* petunia grown for (A) 4 weeks and (B) 7 weeks after transplanting. Results from two different experiments were pooled since there was no block effect. Values shown are means of 12 ± SE.

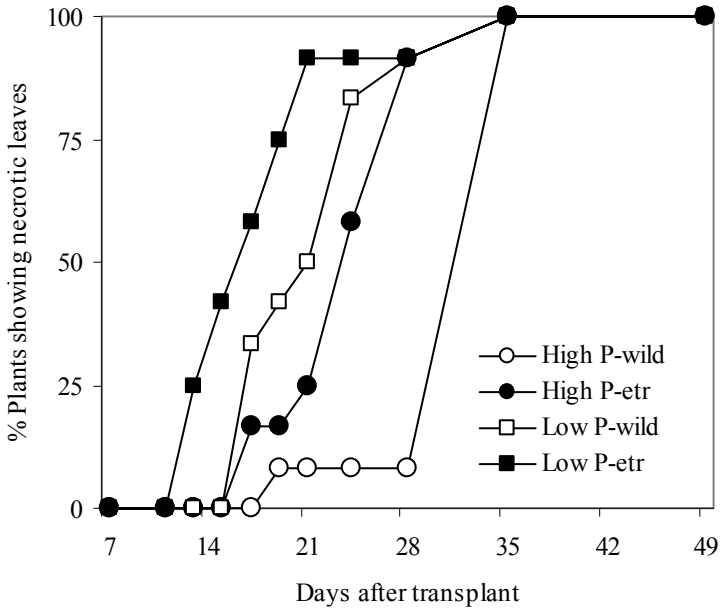


Figure 3.3. Percentage of plants showing disease development symptoms in shoots of wild-type or *etr1-1* petunia during the first 4 weeks after transplanting.

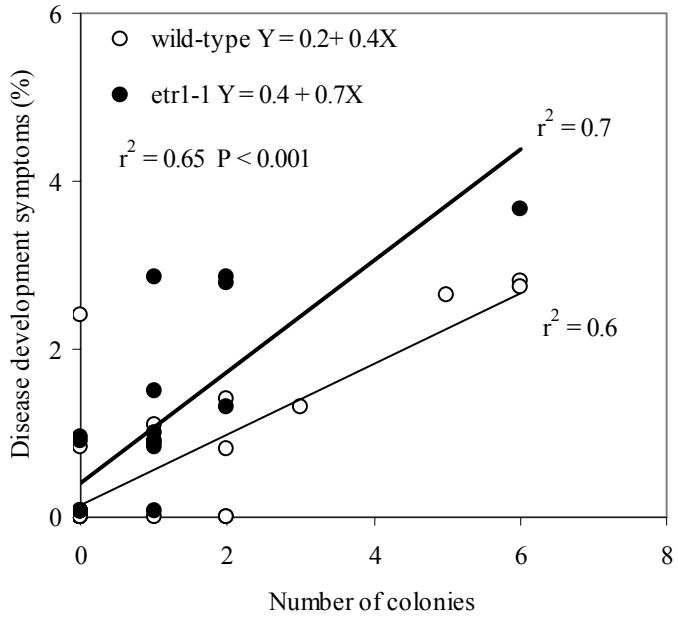


Figure 3.4. The relationship between disease development symptoms in shoots (percentage of necrotic leaves) and the number of colonies developed from roots of plants grown under high phosphorus infected by T. basicola. Root segments harvested from wild-type or *etr1-1* petunia grown for 3 or 4 weeks after transplanting were plated on TB-CEN medium for colony development. Each dot may indicate more than one plant. The slope of the estimated regression function for wild-type petunia was significantly steeper than that for *etr1-1* ($P < 0.001$).

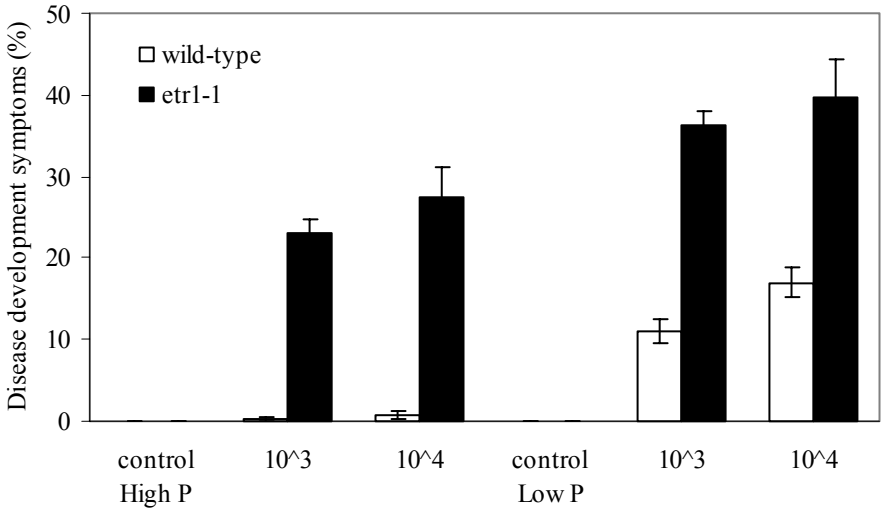


Figure 3.5. The effect of inoculum density (0 (control), $10^3$, and $10^4$ spores/g soil) of *T. basicola* on shoot disease development symptoms as indicated by percentage of necrotic leaves in wild-type and *etr1-1* petunias grown under high or low phosphorus for 4 weeks after transplanting. Each column represents the mean of at least four replicates. Bars indicate standard errors.

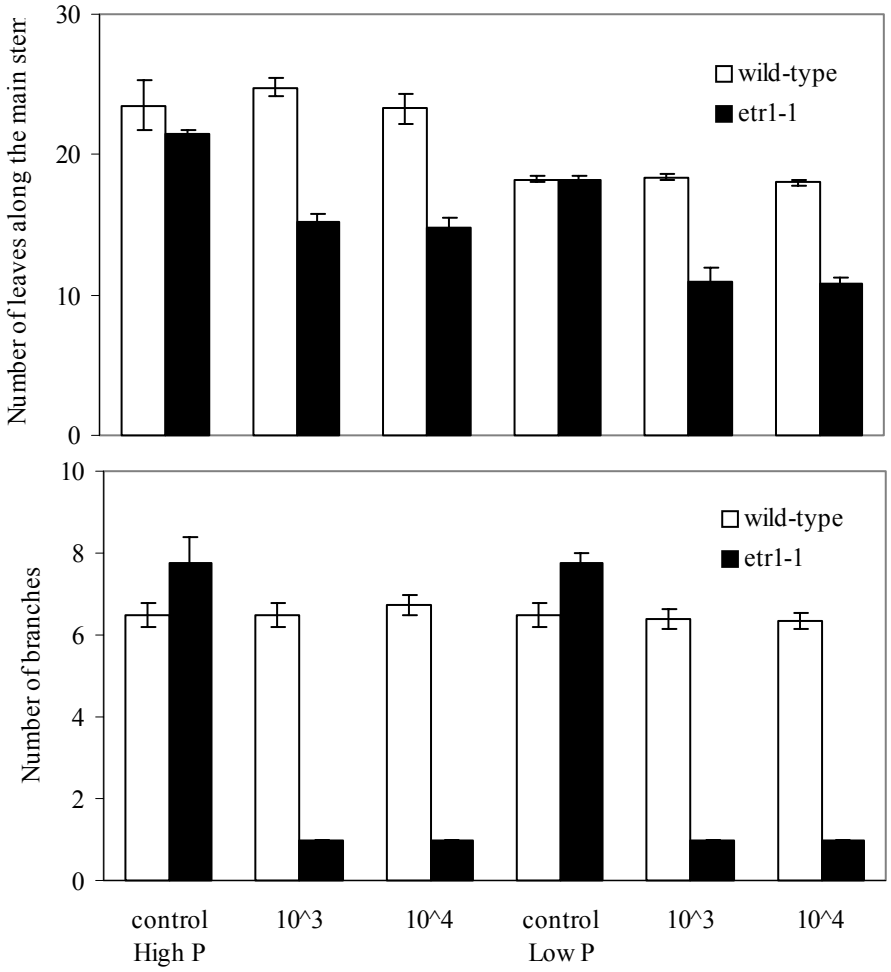


Figure 3.6. The effect of inoculum density (control, $10^3$, and $10^4$ spores/g soil) of *T. basicola* on (A) number of leaves along the main stem and (B) number of branches in wild-type and *etr1-1* petunias grown under high or low phosphorus for 4 weeks after transplanting. Each column represents the mean of at least four replicates. Bars indicate standard errors.

# Chapter 4

## Genetic variation in postproduction quality and ethylene sensitivity of Regal Pelargonium


## ABSTRACT

Ethylene induced petal abscission is a significant problem in Regal Pelargonium during commercial shipping and handling. A genotype of Regal Pelargonium, '99-128-1' has been developed with exceptional production and postharvest characteristics. The individual floret longevity is about twice that of current commercial genotypes. Two progeny also displayed superior floral longevity when compared to commercial genotypes. '99-128-1' and its progeny have significantly reduced ethylene responsiveness resulting in enhanced individual floret longevity. They also have significantly reduced rates of ethylene production. Further, these genotypes exhibited dramatically prolonged whole plant longevity, and displayed more than twice the number of florets than commercial cultivars. Floret longevity of 6 genotypes was strongly correlated with ethylene sensitivity indicated by $S_{50}$ (ethylene concentration for 50% petal abscission), but not with ethylene production, indicating that reduced ethylene responsiveness is an important determinant of enhanced postproduction performance in superior genotypes. Pretreatment with the ethylene action inhibitor 1-methycyclopropene (1-MCP) dramatically decreased petal abscission during simulated transport (3 days, 5 ºC and 95%

RH) in all evaluated cultivars, but the extent of the effect varied with genotype. In all genotypes, petal abscission in response to ethylene increased with floret age. Consequently, 1-MCP had a significant effect on petal abscission of older florets, less effect on freshly opened florets, and no effect on the florets that opened after simulated transport. The longevity of floral display and maximum floret numbers in a postproduction environment were significantly affected by genotype but not by 1-MCP treatment.

## INTRODUCTION

Floral longevity of many species is often terminated by ethylene induced flower senescence or abscission (Abeles et al., 1992). Flowers of the Geraniaceae are particularly sensitive to ethylene, and species within this family were consistent in demonstrating immediate petal abscission in response to applied ethylene (van Doorn, 2001; Woltering and Van Doorn, 1988). Continuous exposure to 1.5 $\mu l \cdot l^{-1}$ ethylene caused complete abscission of petals in ivy geranium within 2 hours (Cameron and Reid, 2001). Petal abscission results from a combination of ethylene synthesis by flower parts and increase of ethylene responsiveness as florets age (Deneke et al., 1990; Evensen, 1991). Ethylene responsiveness occurred when the stigmatic lobes had opened and were receptive to pollination (Evensen, 1991).

Regal Pelargonium (*Pelargonium* x*domesticum* L.H. Bailey) is a flowering potted plant with extraordinary aesthetic characteristics, including abundant and colorful florets, compact growth habit and serrated leaves (Figure 4.1). Postproduction quality of Regal

Pelargonium is greatly reduced by rapid petal abscission within 1 to 2.5 hours after treatment with as little as 1 $\mu l \cdot l^{-1}$ ethylene (Deneke et al., 1990; Olson and Evensen, 1990). Pollination-induced ethylene production resulted in petal abscission within 4 hours of pollination in diploid zonal geraniums (*Pelargonium* x*hortorum*) with single florets (Clark et al., 1997). However, in Regal Pelargonium, the rate of abscission after intentional pollination was low, and abscission rates after simulated shipping did not appear to be related to rates of accidental pollination during shipping (unpublished data). We therefore conclude that the most important aspects of shipping quality for Regal Pelargonium are ethylene production, ethylene exposure (from endogenous or other sources) and ethylene responsiveness. The sensitivity to ethylene also makes this plant difficult to ship while in flower, since the plants abscise petals during shipping and handling. Petal abscission not only greatly reduces the quality of product, but also increases the incidence of gray mold caused by *Botrytis cinerea* when abscised petals fall on the leaves (Cameron and Reid, 2001).

1-methylcyclopropene (1-MCP) has been developed as an inhibitor of ethylene action. It prevents flower abscission in various flowering potted plants (Serek and Sisler, 2001; Serek et al., 1994) as effectively as silver thiosulphate (STS), and it is non-phytotoxic at concentrations higher than those required for maximum effectiveness (Serek et al., 1994; Sisler et al., 1996). However, 1-MCP did not provide additional enhancement of display life of *Campanula*, *Begonia* and *Kalanchoe* in an ethylene-free environment (Serek and Sisler, 2001; Serek et al., 1994) and the effect was transitory since abscission rate of ivy geranium (*Pelargonium* x*peltatum*) increased with time after the application (Cameron and Reid, 2001).

Through the breeding program of the Pennsylvania State University, a genotype of Regal Pelargonium (99-128-1) has been developed with exceptional production and postharvest characteristics. Two progeny from 99-128-1, 00-43-1 and 00-43-2, also had enhanced postproduction quality compared to current commercial genotypes.

The objectives of this study were to compare longevity and postproduction quality of Penn State (PSU) seedlings demonstrating extended floret longevity with that of commercial genotypes. We hypothesized that ethylene production and/or responsiveness might vary among cultivars and that this variation could explain differences in postproduction quality. Further, in an attempt to improve postproduction quality, we treated Regal Pelargoniums with 1-MCP to prevent petal abscission which might occur during shipping and handling.

## MATERIALS AND METHODS

### Plant Material

Rooted cuttings from culture virus-indexed propagative stock of commercial Regal Pelargoniums were obtained from Oglevee Ltd., Connellsville, PA. Cuttings of PSU Accessions, 99-128-1, 00-43-1 and 00-43-2, were taken from stock plants maintained in the Penn State horticulture greenhouses and rooted for 4 weeks in a greenhouse equipped with bottom heat and intermittent mist. Rooted cuttings were placed under natural light supplemented with 110 ± 10 μmol $m^{-2}$ $s^{-1}$ from high intensity discharge metal halide lamps (Sylvania GTE, Manchester, NH, USA) for 4 weeks to stimulate floral initiation. Photosynthetically active radiation varied throughout the day

with a maximum of 1400 µmol $m^{-2}s^{-1}$. Day and night temperatures in the greenhouse during the experimental period were 21/16 ºC with an 18 h photoperiod. The plants were potted in 15 cm (1.5 L) azalea pots with Aggregate Plus Media – Sunshine Mix-4 (SUN GRO Horticulture, Bellevue, Washington) and placed under the same light scheme as described above. The plants were alternately fertilized at each irrigation with Peters Professional Foliar Feed, 27-15-12, and Miracle-Gro Professional Excel, 15-5-15 CAL-MAG (Scotts-Sierra, Marysville, Ohio). Foliar sprays of insecticidal soap were used before anthesis to control whitefly populations.

Florets were labeled at the day of anthesis with small paper tags to record the date. Floret longevity, defined as the number of days between anthesis and senescence (petal abscission), was evaluated using florets on intact plants in the greenhouse. The date of flower senescence was recorded when 50 % or more petals were abscised in response to a gentle touch.

For the evaluation of whole plant longevity, plants of the same age were used. All open florets were removed from each plant about two weeks before the commencement of the experiment. When new florets began to open, the florets were labeled with paper tag to record the date of anthesis until the plant bore a population of 0 to 10 day-old florets. Senescent florets were removed from the plant when petals were abscised by a gentle stroke before the experiment. The plants were then used for 1-MCP treatment and subsequent evaluation of postproduction longevity as described below.

**Evaluation of ethylene responsiveness**

Individual florets of known ages were harvested from plants grown in the greenhouse, and placed in a plastic rack inside a plastic container with the pedicels in distilled water. The container was sealed after the water level was adjusted to produce a standard container gas volume. Ethylene gas was injected into the chamber to provide the desired concentration, and the container was kept at 22.5 ± 0.1ºC for 90 minutes. Ethylene concentrations were analyzed by sampling with 1 $cm^3$ syringes from the head space of the sealed container, and the concentration was determined using a gas chromatograph Hewlett-Packard 6890, Palo Alto, CA, USA) fitted with a flame ionization detector and an activated alumina column. The detection limit was 0.013 $cm^3$ $m^{-3}$. The lid was opened and petal abscission was evaluated 1 h after completion of ethylene treatment. Abscission rate was calculated based on the proportion of total petals shed when the florets were shaken lightly. The first set of experiments were conducted from February to March 2002 (season 1), and the second set of experiments from December 2002 to January 2003 (season 2). Each treatment included at least 6 florets of each age.

**Ethylene production measurement**

Ethylene production of florets was measured by enclosing each floret in a 5 ml vial. Each floret with petals attached was rolled gently and put inside a vial. After one hour incubation at 22.5 ± 0.1ºC, ethylene was sampled with 1 $cm^3$ syringes from the head space of the sealed vials and the concentration was determined by gas chromatography as

described. Ethylene production (nl/g FW/h) was calculated on the basis of fresh weight of each sample. Sample fresh weight was recorded before the ethylene measurement.

**1-MCP and ethylene treatment**

1-MCP (trade name: EthylBloc) was obtained from Floralife Inc, Walterboro, SC. Three plants in full bloom were placed in a 106 × 74 × 64 cm (126 L) plastic container situated in the laboratory. After the container was sealed, 1 $\mu l \cdot l^{-1}$ 1-MCP was applied to the plants based on the methods described by Cameron and Reid (2000). The EthylBloc powder was placed in a weighing boat suspended from the ceiling of the container. Buffer solution was injected through a septum with a syringe to release 1-MCP. Plants were treated with 1-MCP for 4 hours at 22.5 ± 0.1ºC, 50 ± 15% RH with 10 ± 0.2 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool-white fluorescent lamps. The container was opened for ventilation and resealed for the treatment of 0.3 $\mu l \cdot l^{-1}$ ethylene for 4 hours. Plants were then removed from the container and placed in simulated consumer environment (SCE) for evaluating postproduction performance as described below. The experiment was carried out from March to May 2003.

**1-MCP and simulated transport**

The 1-MCP treatment was conducted in the greenhouse as described above. The temperature inside the plastic container was 20.4 ± 0.02 ºC. After the 1-MCP treatment, plants were placed in plastic sleeves and moved to a refrigerator maintained at 5 ºC and 95% RH to simulate transport for 3 days in darkness. Ethylene samples were collected at four different locations in the airspace of the refrigerator during simulated transport and

0.067 ± 0.004 $\mu l \cdot l^{-1}$ethylene was detected. Plants were then moved to SCE for evaluating postproduction quality as described below. This experiment was conducted from December 2002 to January 2003.

**Evaluation of postproduction longevity**

To evaluate whole plant longevity, plants bearing various ages of florets were moved to a SCE. The SCE was maintained at 22.5 ± 0.1 C, 50 ± 15 % RH, and 40 ± 0.5 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool-white fluorescent lamps from 0800 to 2000 HR daily. Temperature, relative humidity and photosynthetic photon flux was measured at midshoot height and recorded by the HOBO data logger (Onset Computer Corporation, Bourne, MA). Plants were irrigated as needed with distilled water.

Dates of floret anthesis and senescence of each floret were recorded on paper tags before, during and after simulated transport to determine individual floret longevity. Florets were considered senescent if they wilted or if ≥ 50 % of the petals had abscised. The whole plant longevity was defined as the number of days from placement in the SCE until five or fewer healthy florets remained on the plant. Number of open florets at each week and maximum floret number were calculated on the basis of the dates recorded on the paper tag. At least four plants of each genotype were evaluated for individual floret longevity and whole plant longevity.

**Statistical analysis**

Analysis of variance (ANOVA) and mean separations (Fisher's PLSD test) were conducted using StatView (SAS Institute) and were considered significant at $P < 0.05$.

The curve describing ethylene responsiveness (ethylene concentration vs. abscission) was fitted by SigmaPlot (SigmaPlot 8.0, SPSS Inc.) with regression analysis. An F-test was performed to analyze whether each age group was significantly different from the others in abscission rate.

## RESULTS

### Longevity of individual florets

When the plants were grown in the greenhouse, the PSU Accessions 99-128-1 and 00-43-2 displayed about twice the floral longevity of other genotypes (Figure 4.2). The floret longevity of 00-43-1 was also higher than that of other genotypes, while 'Maiden Lilac' displayed shortest floral longevity among the evaluated genotypes.

### Endogenous ethylene production

Ethylene production rates of excised florets of Regal Pelargonium were quite low, ranging from 0.1 to 1.4 nl/g FW/h (Figures 4.3 and 4.4). Regardless of genotype, ethylene production was highest on the day of anthesis and decreased within a week, and demonstrated a climacteric-like pattern as the floret aged (Figure 4.3).

We evaluated a larger number of genotypes during the first 6 days after anthesis. Figure 4.4 indicates ethylene production rates of the genotypes, which were categorized by floret longevity or ethylene production pattern. Freshly opened florets of 00-43-1 produced significantly higher ethylene on the day of anthesis than other genotypes (Figure 4.4.A.). 'Ballet' and 'Emperor', which display relatively short floret longevity

(Figure 4.2), produced the lowest rate of ethylene on the day of anthesis and the ethylene production rate remained low during the first 6 days post-anthesis (Figure 4.4.D.). There was no correlation between ethylene production rates and individual floret longevity on intact plants either on the day of anthesis or three days after anthesis, when stigmatic lobes begin to separate (Figure 4.5).

**Ethylene responsiveness**

Variation in petal abscission was observed among genotypes when the florets of various ages were exposed to 0.015 $\mu l \cdot l^{-1}$ ethylene for 90 minutes in season 1 (Table 4.1). 99-128-1 and 00-43-2 were not responsive to this concentration of exogenous ethylene, and 00-42-1 demonstrated an intermediate response. All genotypes except 99-128-1 and 00-43-2 responded to ethylene as low as 0.015 $\mu l \cdot l^{-1}$ with a high rate of petal abscission. Substantial abscission occurred even in freshly opened florets of 'Ballet' exposed to 0.015 $\mu l \cdot l^{-1}$ ethylene. The abscission rate following ethylene treatment increased with floret age (Table 4.1).

In season 2, 3 to 5 day-old florets of each genotype were treated with a range of ethylene concentrations to generate dose-response curves. Petal abscission rate sharply increased over a narrow ethylene concentration rage in most of genotypes (Figure 4.6-1). The data within floret age classes of 99-128-1 and 00-43-2 were more scattered than those of the other genotypes (Figure 4.6-1). When the abscission rate was natural log-transformed, abscission rate was linearly related to the ethylene concentration (Figure 4.6-2), and PSU genotypes, 99-128-1 and 00-43-2, had no difference in abscission rate among age classes. The petal abscission rate of commercial genotypes significantly

increased as florets aged, while the rate was high in 'Maiden Lilac' irrespective of the floret age (Figure 4.6-2). Three day-old florets of 99-128-1, 00-43-2 and 00-43-1 did not respond to ethylene concentrations lower than 0.4 $\mu l \cdot l^{-1}$ for 90 minutes (Figure 4.7) and demonstrated significantly reduced petal abscission rate when they were exposed to the same amount of ethylene as other genotypes.

The responsiveness coefficients (Nissen, 1985) were used to analyze ethylene responsiveness of various genotypes. In season 2, the PSU Accessions had high values of $S_{10}$, $S_{50}$ and $S_{90}$ (the ethylene concentration for 10%, 50% and 90% abscission, respectively) relative to other genotypes (Table 4.2). Although $S_{90}$ was also high in those genotypes, the increase in $S_{10}$ proportionally decreased the ratio $S_{90}/S_{10}$ (Table 4.2). When the experiment was conducted in a different season (Table 4.2, season 1), the order of the genotypes remained almost the same among S-coefficients, but the ratio $S_{90}/S_{10}$ varied between seasons (Table 4.2). Floret longevity was correlated with the $S_{50}$ of 3 day old florets, which was increased in season 2 (Figure 4.8).

**Effects of 1-MCP on postproduction performance after ethylene treatment**

The PSU Accessions developed and maintained more than twice number of florets compared to other genotypes in the simulated consumer environment (Figure 4.9). The PSU Accessions retained florets after 3 weeks in the SCE, while other genotypes had abscised all their florets by then (Figures 4.9 and 4.10). In particular, the genotype 99-128-1 held more than 100 florets even after 3 weeks in the SCE (Figure 4.9). Whole plant longevity was significantly better for 99-128-1, 00-43-1 and 00-43-2 than for other genotypes (Figure 4.10) and was not increased by 1-MCP (data not shown). The number

of florets on 1-MCP treated plants was equal to that of control plants even after ethylene exposure (Figures 4.11 and 4.12). Without 1-MCP, ethylene reduced floret number differently among genotypes, but ethylene effects were only observed immediately after treatment (Figures 4.11 and 4.12). The reduction in floret longevity was significant in very-old and old florets (Figure 4.13). The longevity of freshly opened florets was also reduced by ethylene treatment in all genotypes except 99-128-1. 1-MCP did not increase either the number of florets or individual floret longevity, but only protected plants from the effects of ethylene (Figures 4.11 and 4.13).

**Effects of 1-MCP on postproduction performance after simulated transport**

Ethylene-induced petal abscission during simulated transport varied among genotypes (Figure 4.14) and was lowest in 99-128-1 and 00-43-2. The longevity of florets older than 3 days was dramatically reduced during simulated transport in all the evaluated genotypes (Figure 4.15). 1-MCP pretreatment inhibited petal abscission during simulated transport in all the genotypes (Figures 4.14 and 4.15). The effect of 1-MCP on enhancement of floret longevity was more pronounced in older florets (Figure 4.15). In ‘Maiden Orange’, the longevity of all groups of florets open before simulated transport was dramatically enhanced by the 1-MCP treatment (Figure 4.15.D.). However, the longevity of the “fresh” and “open during” floret groups was not improved by the 1-MCP treatment in the other three genotypes (Figure 4.15.C). The longevity of florets open after 1-MCP treatment was not influenced by 1-MCP in any of the genotypes (Figure 4.15).

The PSU Accessions retained a higher number of florets in the SCE compared to other genotypes and displayed significantly higher maximum floret number (Figures 4.16

and 4.16). 99-128-1 had greater whole plant longevity compared to other genotypes (data not shown). 1-MCP treatment did not affect any of those variables (Figures 4.16, and 4.17). The whole plant longevity and number of florets at 2 weeks in the SCE were strongly correlated with individual floret longevity on intact plants (Figure 4.18).

## DISCUSSION

### 99-128-1 and its progeny, 00-43-2, displayed superior postproduction performance in comparison with other genotypes.

Our results clearly demonstrate that postproduction quality of the Regal Pelargoniums varies significantly among genotypes. The genetic differences in postproduction quality were consistent even when the experiment was conducted in different seasons, indicating stable genetic effects for the characteristic.

PSU genotypes displayed exceptional postproduction performance when compared to commercial cultivars. They had dramatically prolonged whole plant longevity and maintained higher number of florets in the SCE (Figures 4.9, 4.10, 4.11 and 4.16). When the experiment was conducted in spring, the PSU genotypes had much higher number of florets even at the commencement of the experiment (Figure 4.9), although all the genotypes were at the same developmental stage. Therefore, increased whole plant longevity of those genotypes would be attributable to the greater individual floret longevity and the formation of higher number of florets (Figure 4.18).

Ethylene exposure significantly increased petal abscission in all the genotypes (Figures 4.11 and 4.12), however, floret number was equal among treatments 1 week

later (Figures 4.11). The reason is that the flower buds were insensitive to ethylene and were not shed after ethylene exposure, and those buds opened in the simulated consumer environment, replacing senescent florets.

**Significantly reduced ethylene responsiveness contributes to the superior postproduction performance of PSU genotypes.**

Regal Pelargonium is classified as one of the ornamental species most sensitive to ethylene (Deneke et al., 1990; Woltering, 1987). All of the Regal Pelargoniums that we examined responded to ethylene, however, ethylene responsiveness varied significantly among genotypes. Commercial genotypes displayed significant petal abscission in response to ethylene treatment at concentrations as low as 0.015 $\mu l \cdot l^{-1}$ in one season (Table 4.1) but required higher concentrations of ethylene in the other season (Table 4.3), indicating seasonal variation in this response. Ethylene-treated 'Ballet' florets abscised petals in response to low ethylene concentrations even on the day of anthesis. This concentration is extremely low compared to what has been utilized in other experiments (Cameron and Reid, 2001; Evensen, 1991; van Doorn, 2001; Woltering, 1987; Woltering and Van Doorn, 1988) and demonstrates the reason for the poor shipping performance of most genotypes of Regal Pelargonium. Commercial genotypes could easily abscise petals even in the presence of very small amounts of ethylene resulting from stresses imposed during shipping or handling, or in response to exogenous ethylene.

To investigate the cause of the superior postproduction quality of 99-128-1, we measured the endogenous ethylene production and ethylene responsiveness of florets at different floret ages. Although there was no clear relationship between the cumulative

amount of ethylene production in excised florets and the longevity of florets on intact plants (Figure 4.5), the timing of a climacteric rise in ethylene production preceded petal abscission reflecting differences in floret longevity of the evaluated genotypes (Figure 4.3).

Dose response data indicated that 99-128-1 was significantly less sensitive to ethylene than any other genotype evaluated (Figures 4.6 and 4.7). Even with 1 $\mu l \cdot l^{-1}$ ethylene exposure, petal abscission of 3 day old 99-128-1 remained lower than 50% (Figure 4.7). The differences in ethylene responsiveness among the genotypes can explain much of the genetic variation in postproduction quality in Regal Pelargoniums. 99-128-1 and its progeny 00-43-2 displayed extended floret longevity, which was strongly associated with reduced ethylene responsiveness (Figure 4.8). Therefore, our results clearly demonstrate that ethylene responsiveness plays a critical role in regulating petal abscission and floral longevity in Regal Pelargoniums, and that the PSU genotypes have significantly prolonged floral longevity because of lower ethylene responsiveness.

Quantification of ethylene responsiveness with responsiveness coefficients indicated that the PSU Accessions, like commercial cultivars, are also classified ultraresponsive despite their significantly reduced ethylene responsiveness. The $S_{10}$, $S_{50}$, and $S_{90}$ values allow direct comparison of ethylene responsiveness among genotypes and indicate the absolute values of ethylene concentration to which these plants respond, while responsive coefficient ($S_{90}/S_{10}$) indicates the slope of the main part of the response curve, indicating how narrow is the range over which responsiveness occurs, without informing where that curve lies in terms of absolute amounts of ethylene. Therefore,

higher responsive coefficient ($S_{90}/S_{10}$) was not related to less ethylene responsiveness in this study with various genotypes.

What accounts for the reduced ethylene responsiveness in 99-128-1? Florets of this genotype take 1 or 2 days longer than other genotypes to progress from anthesis to separation of stigmatic lobes. Separation of the stigmatic lobes signifies receptiveness to pollination and the development of ethylene responsiveness (Evensen, 1991). Further, the slope of the ethylene response curve for 99-128-1 was much lower than that of other genotypes (Figure 4.7), and the data were scattered more than any other genotypes. These results support the hypothesis that delayed development of ethylene responsiveness could partly explain the reduced ethylene responsiveness of 99-128-1. Changes in ethylene sensitivity may be mediated by modulation of receptor levels during development (Payton et al., 1996; Trewavas, 1982) and the ethylene receptor ETR is a negative regulator of ethylene response (Hua and Meyerowitz, 1998). The increase in $S_{10}$ and the reduced slope of dose response curve for 99-128-1 (Figure 4.7) indicate that it has much lower affinity or much higher number of ethylene binding sites to be saturated, and/or it may have alterations in ethylene signal transduction pathway (Chang and Shockey, 1999). In view of the seasonal changes in S-coefficients demonstrated in our experiment (Table 4.2), the response mechanism appears to be modified by environmental conditions that could hasten or delay plant developmental process.

Our results demonstrate that there is large variation in ethylene responsiveness among genotypes of Regal Pelargonium, and that the enhanced postharvest performance of PSU Accessions is derived from reduced ethylene responsiveness of individual florets. The Penn State Accessions were developed by conventional breeding in an effort to

improve disease and insect resistance, to continue flowering under high temperature, and to improve postproduction quality. In our experiment, some genotypes were identified with superior postproduction performance related to reduced ethylene responsiveness of individual florets. Therefore, genotypes with improved postproduction quality could be identified by selection for reduced ethylene responsiveness of florets. Regal Pelargoniums are propagated only by rooted cuttings since the majority of them are putative tetraploids, therefore, seedling production is not presently feasible due to low seed numbers and germination rates (Craig, 1982). However, eariler identification of superior postproduction characteristics would be more desirable if it is possible. In seed-propagated geraniums, seedlings were evaluated in response to ethylene to identify cultivars with reduced sensitivity, and their hypocotyl elongation was correlated with petal abscission of florets (Clark et al., 2001). Therefore, it would be interesting to see whether there is a correlation between these characteristics in Regal Pelargoniums.

**1-MCP inhibited petal abscission during simulated transport, but the efficacy varied with genotypes.**

Pretreatment with 1-MCP effectively inhibited petal abscission in the presence of ethylene in all the evaluated genotypes. However, the efficacy of 1-MCP varied with genotype. Similar results were reported in zonal geraniums, in which multiple genotypes were used to evaluate 1-MCP effect and petal abscission was not completely inhibited in the most ethylene sensitive cultivar by the 1-MCP pretreatment (Jones et al., 2001). The cause of such variation in 1-MCP effectiveness among cultivars is unknown. However, it

is not unusual in light of the genetic variations in endogenous ethylene production rate and ethylene responsiveness among the genotypes.

In our experiment, the floret longevity of ‘Maiden Orange’ displayed significant enhancement by the 1-MCP treatment, while ‘Maiden Rose Pink’ demonstrated less improvement in floret longevity after 1-MCP treatment (Figure 4.15). Although an increase in 1-MCP dosage did not provide additional benefit in preventing petal abscission of ivy geraniums (Cameron and Reid, 2001; Serek and Sisler, 2001), prolonging treatment time with 1-MCP inhibited petal abscission of the sensitive cultivar to the level of control in zonal geraniums (Jones et al., 2001). Therefore, an increase in treatment duration may enhance the longevity of ‘Maiden Rose Pink’ florets that have opened before the simulated transport.

**1-MCP effects vary among floret ages.**

Pretreatment with 1-MCP effectively inhibited petal abscission of florets older than 3 days during simulated transport, improving longevity of those florets. The effect of 1-MCP significantly varied among floret ages and the 1-MCP treatment did not improve the longevity of florets that were too young at the time of treatment. Those results can be explained by the fact that ethylene sensitivity changes as floret ages and that young florets have not yet acquired full sensitivity (Deneke et al., 1990; Evensen, 1991). 1-MCP did not improve the longevity of near-senescent florets (data not shown) as was demonstrated in miniature rose and Oriental hybrid lilies (Celikel et al., 2002; Muller et al., 2001). Therefore, the maximum effectiveness of 1-MCP treatment could be achieved

by treating Regal Pelargoniums when they bear florets older than 3 days. Near-senescent florets should be removed prior to shipping.

While silver ions remain in plant tissue and bind to new binding sites as they are synthesized, giving long-term protection from ethylene (Veen, 1983), 1-MCP did not protect florets that opened after simulated transport and did not affect the maximum floret number or the longevity of whole plants. Those results support the hypothesis that 1-MCP has a transitory effect which might be due to the fact that new ethylene binding sites are continuously synthesized as new florets subsequently develop, leaving those florets unprotected. Sufficient ethylene to induce abscission could easily accumulate in floral distribution centers and retailers. Repeated application of 1-MCP could reestablish the protection of florets opening after 1-MCP treatment, but this is not practical (Cameron and Reid, 2001). Regardless of 1-MCP treatment, both 99-128-1 and 00-43-2 displayed twice the maximum floret number as Maiden Orange and Maiden Rose Pink, and the longevity of whole plants was also significantly extended (Figures 4.10 and 4.17). Therefore, pretreatment with 1-MCP is beneficial to protect from ethylene exposure during shipping, but genetic enhancement protects flowers from ethylene during the whole life of the plant.

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Table 4.1. Effects of genotype and floret age on ethylene-induced petal abscission (Season 1). Excised florets were exposed to 0.015 $\mu l \cdot l^{-1}$ ethylene for 90 min. Each point represents the average percent abscission of at least 6 florets.

| Floret age (days) | 99-128-1 | 00-43-2 | 00-43-1 | Maiden Orange | Maiden Lilac | Baroness | Ballet |
|---|---|---|---|---|---|---|---|
| 0 | 0% | 0% | 0% | 0% | 0% | 0% | 47% |
| 1 | 0% | 0% | 0% | 30% | 0% | 53% | 100% |
| 2 | 0% | 0% | 0% | 38% | 83% | 100% | 100% |
| 3 | 0% | 0% | 20% | 48% | 87% | 100% | 100% |
| 4 | 0% | 0% | 27% | 54% | 90% | 100% | 100% |
| 5 | 0% | 0% | 40% | 100% | 93% | 100% | 100% |
| 6 | 0% | 0% | 60% | 100% | 100% | 100% | 100% |

Table 4.2. Effect of genotype and season on ethylene responsiveness. Excised florets were treated with various concentrations of ethylene for 90 min.

| Season | Genotype | Ethylene ($nl\ l^{-1}$) for proportion of response | | | Responsiveness coefficient |
|---|---|---|---|---|---|
| | | $S_{10}$ | $S_{50}$ | $S_{90}$ | $S_{90}/S_{10}$ |
| 1 | 99-128-1 | 995 | 1316 | 1616 | 1.62 |
| (Feb~Mar) | 00-43-2 | 81 | 384 | 594 | 7.30 |
| | 00-43-1 | 11 | 114 | 272 | 23.95 |
| | Maiden Rose Pink | 14 | 50 | 103 | 7.36 |
| | Maiden Lilac | 5 | 15 | 28 | 5.28 |
| | Maiden Orange | 1 | 6 | 20 | 21.95 |
| 2 | 99-128-1 | 577 | 1054 | 1325 | 2.3 |
| (Dec~Jan) | 00-43-2 | 507 | 724 | 903 | 1.8 |
| | 00-43-1 | 561 | 636 | 683 | 1.2 |
| | Maiden Rose Pink | 73 | 240 | 422 | 5.8 |
| | Maiden Lilac | 51 | 111 | 175 | 3.4 |
| | Maiden Orange | 73 | 121 | 164 | 2.2 |

Season 2 was taken from the data in Figure 6.

$S_{10}$ : the ethylene concentration for 10% abscission

$S_{50}$ : the ethylene concentration for 50% abscission

$S_{90}$ : the ethylene concentration for 90% abscission

$S_{90}/S_{10}$ : a measure of the spread of the dose response curve

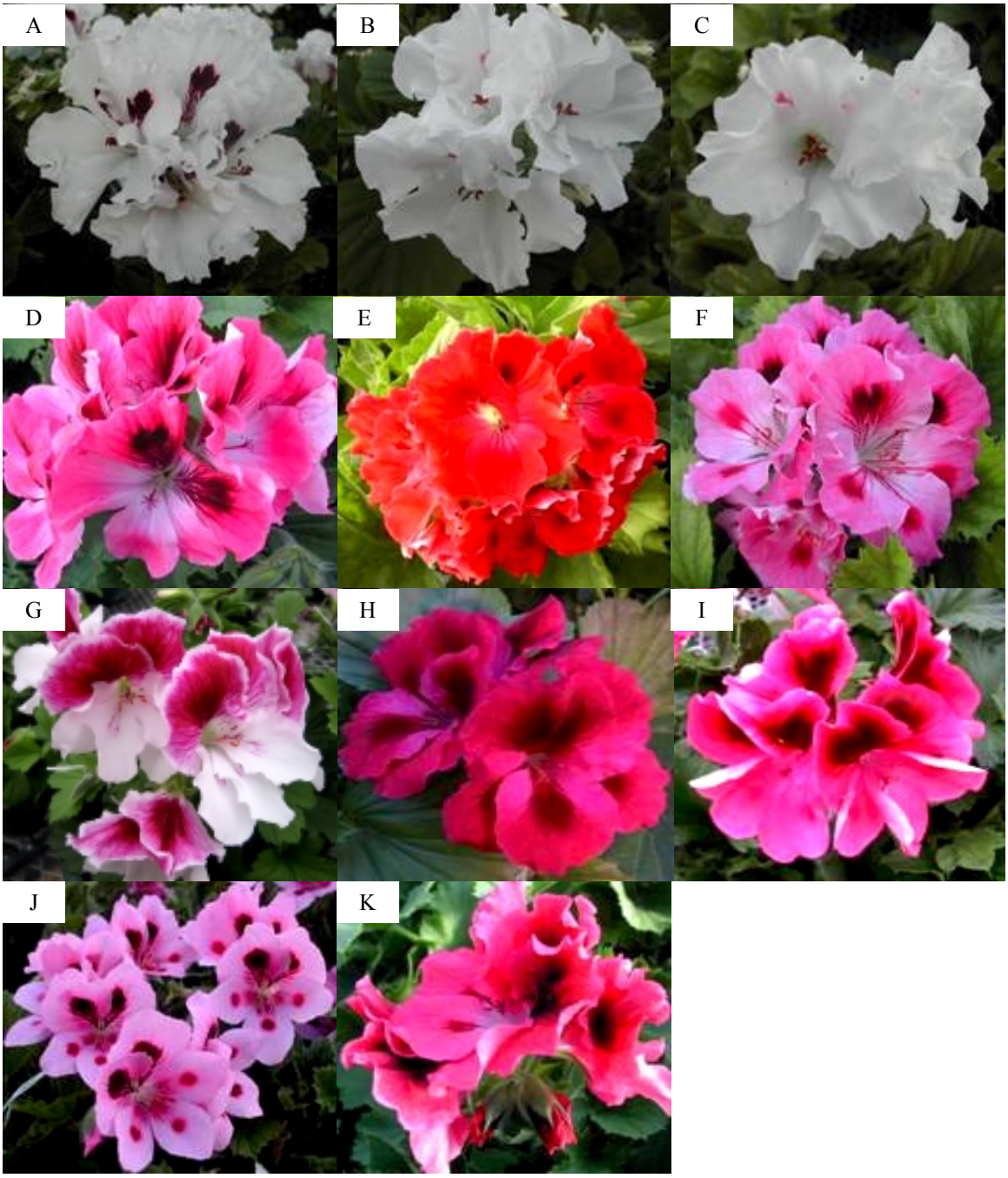


Figure 4.1. Inflorescences of Regal Pelargoniums used in this experiment: Penn State Accessions (A) 99-128-1, (B) 00-43-1 and (C) 00-43-2, and commercial cultivars (D) ‘Maiden Rose Pink’, (E) ‘Maiden Orange’, (F) ‘Maiden Lilac’, (G) ‘Bravo’, (H) ‘Baroness’, (I) ‘Dandy’, (J) ‘Emperor’, and (K) ‘Ballet’.

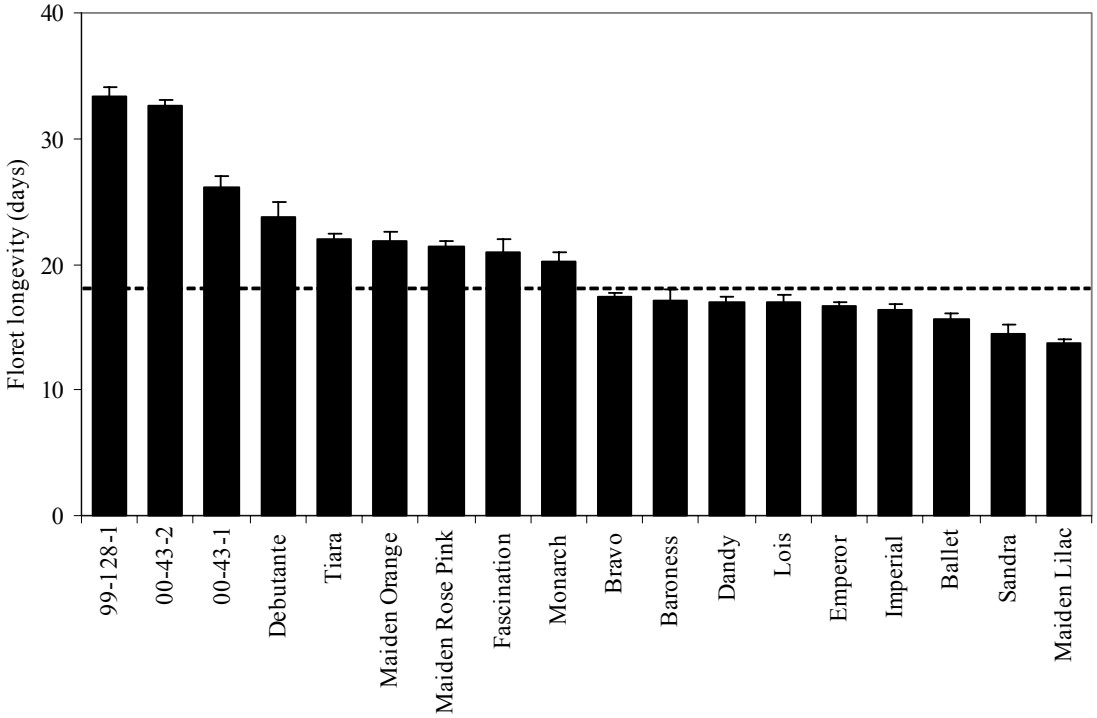


Figure 4.2. Floral longevity of 18 genotypes evaluated on intact plants in the greenhouse. The dotted horizontal line indicates the average floret longevity of the commercial genotypes. Data shown are means of at least 10 florets ± SE.

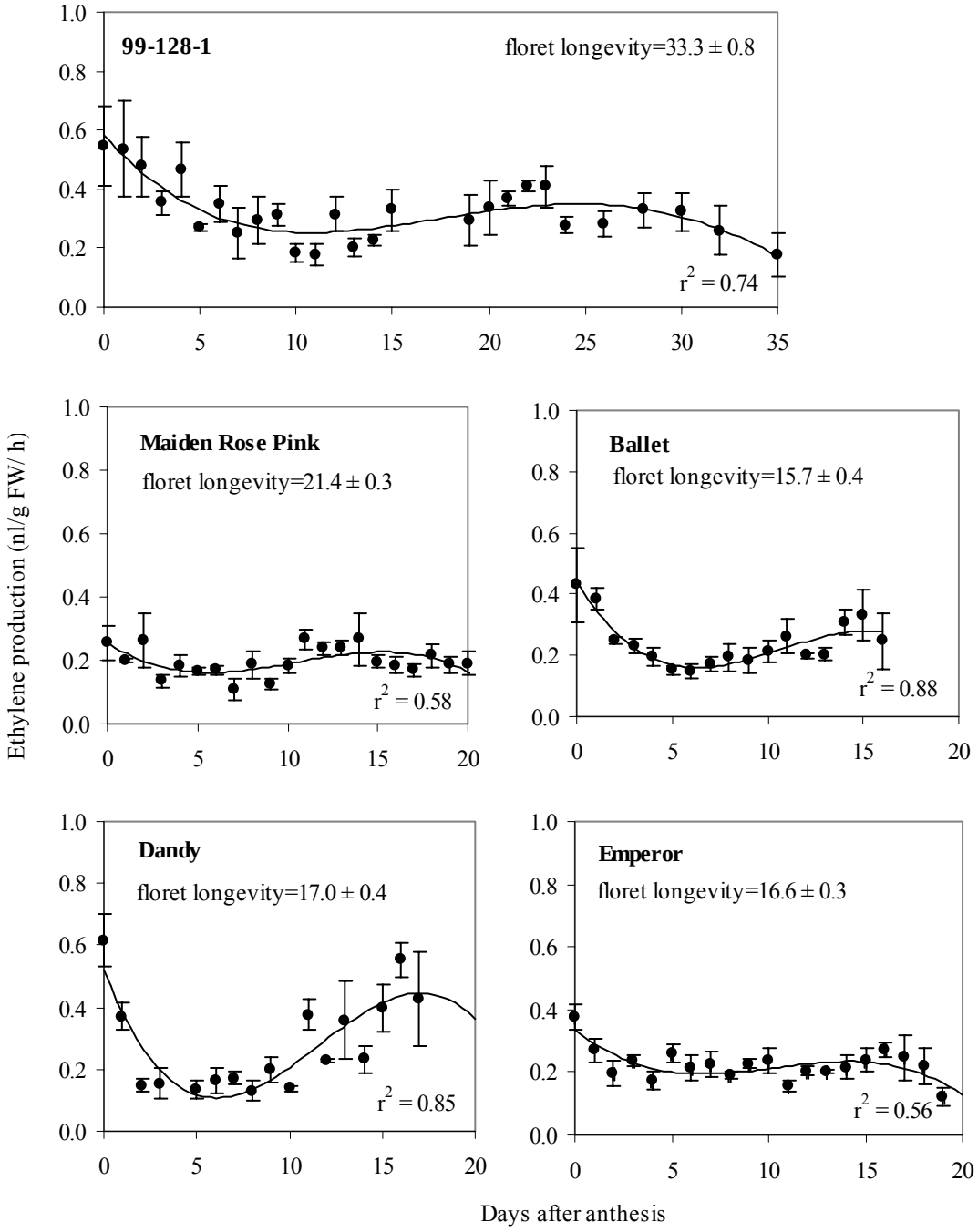


Figure 4.3. Relationship between floret age and ethylene production by freshly-excised florets of 5 genotypes of Regal Pelargonium. Data shown are means of at least 4 florets ± SE. Note variation in scale of x-axis reflecting differences in floret longevity.

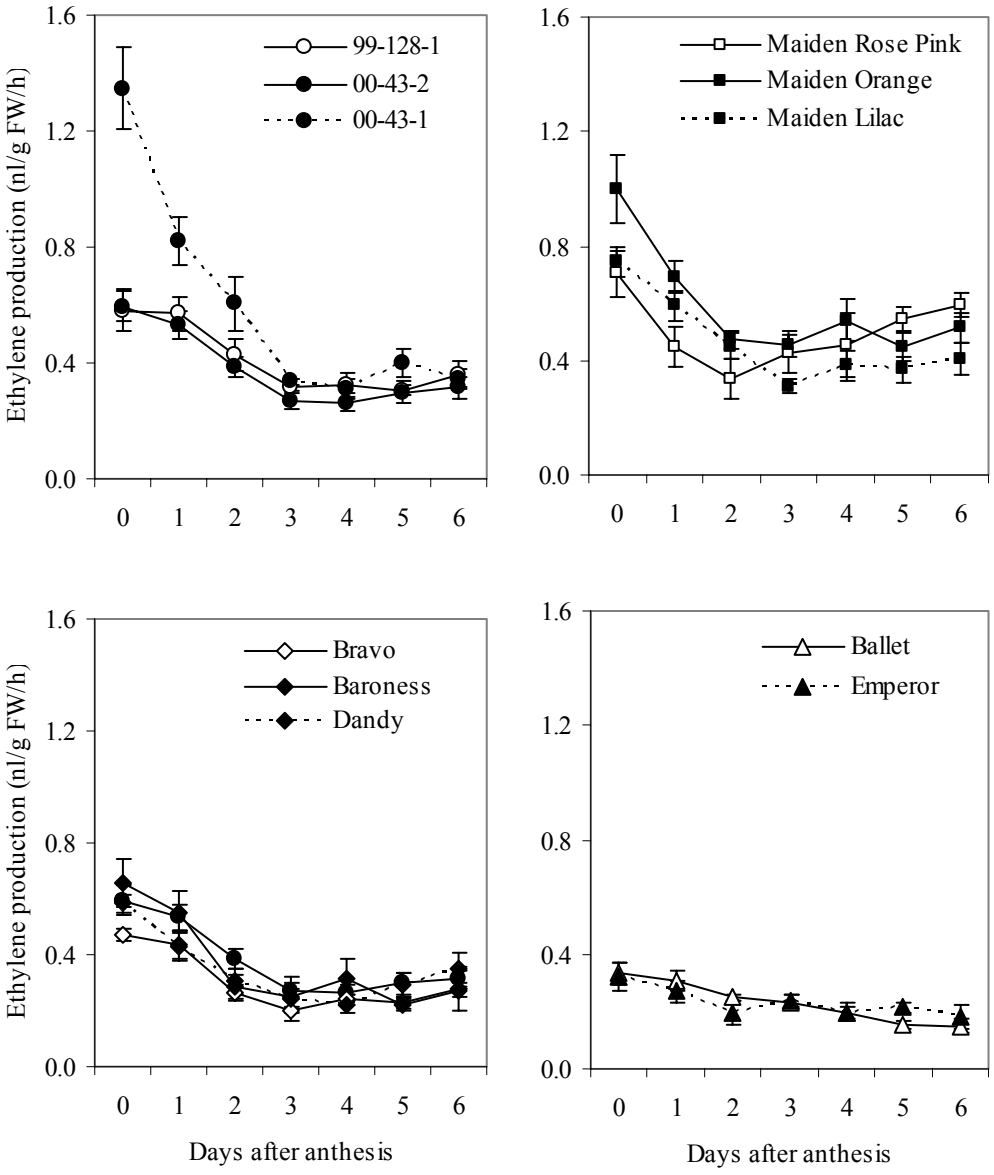


Figure 4.4. Ethylene production of freshly excised florets during the first 6 days after anthesis. Data shown are means of at least 5 florets ± SE. The genotypes were categorized by floret longevity or ethylene production pattern.

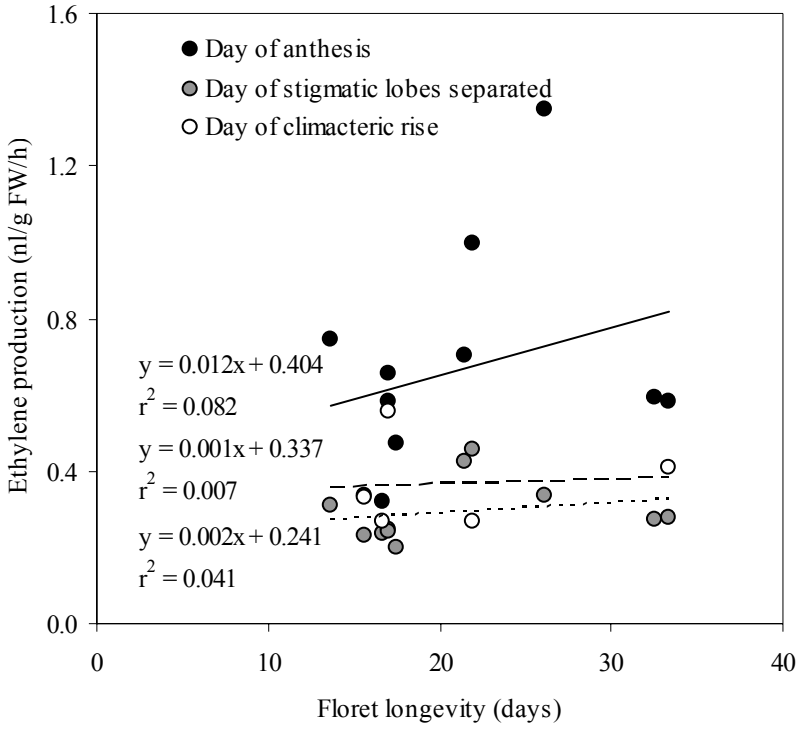


Figure 4.5. There was no significant Relationship between individual floret longevity and ethylene production. The ethylene production of excised florets harvested on the day of anthesis, when stigmatic lobes had separated, or at the climacteric peak was plotted against floret longevity on the intact plant. All the genotypes shown in Figure 4.4 were included in this figure.

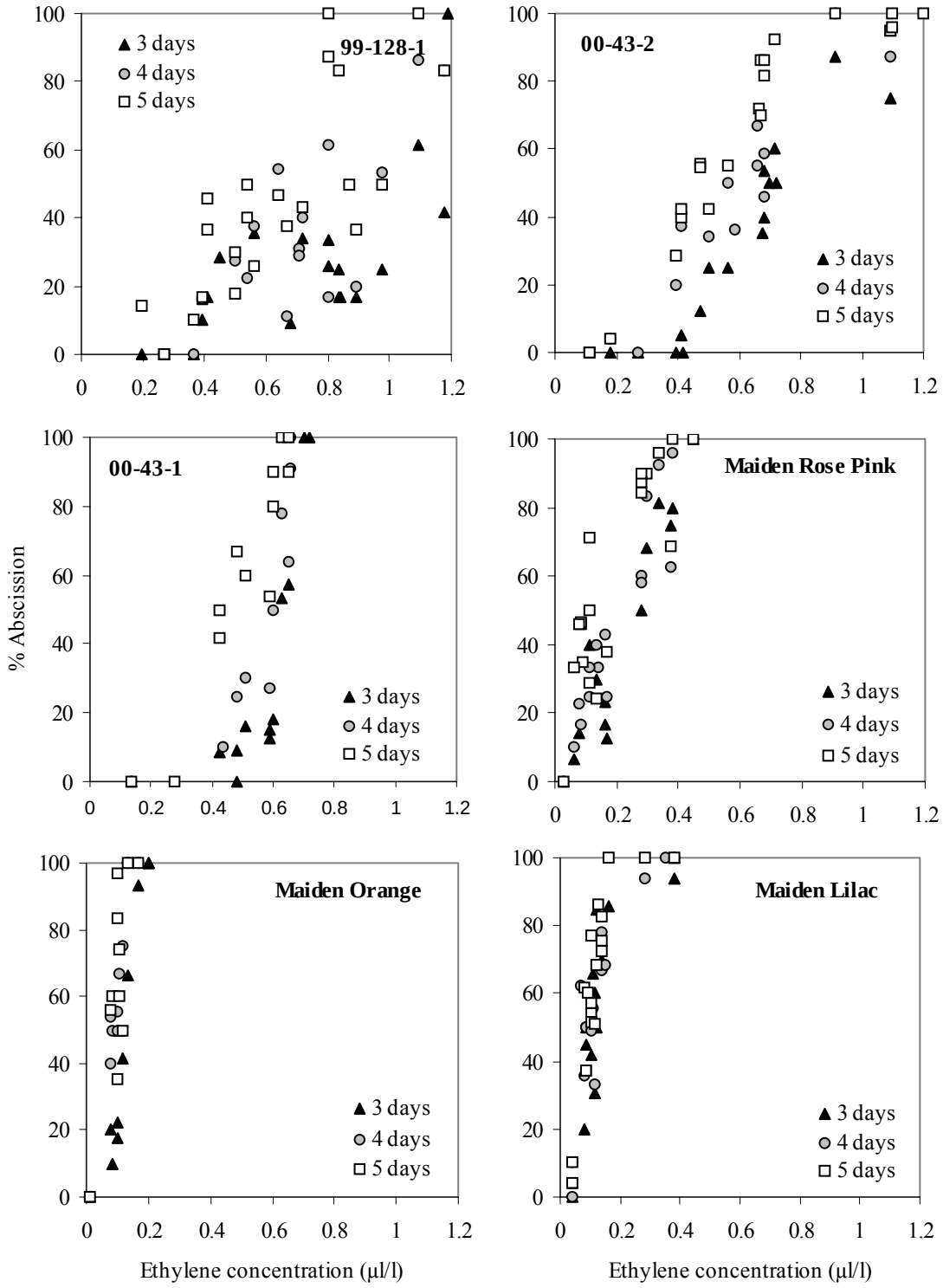


Figure 4.6-1. The effect of ethylene concentration on petal abscission for 3-, 4- and 5-day old florets (Season 2). Excised florets were exposed to ethylene for 90 minutes. Each point represents the percent abscission of at least 6 florets. The variables floret age and ethylene concentration significantly affected petal abscission at $P<0.001$.

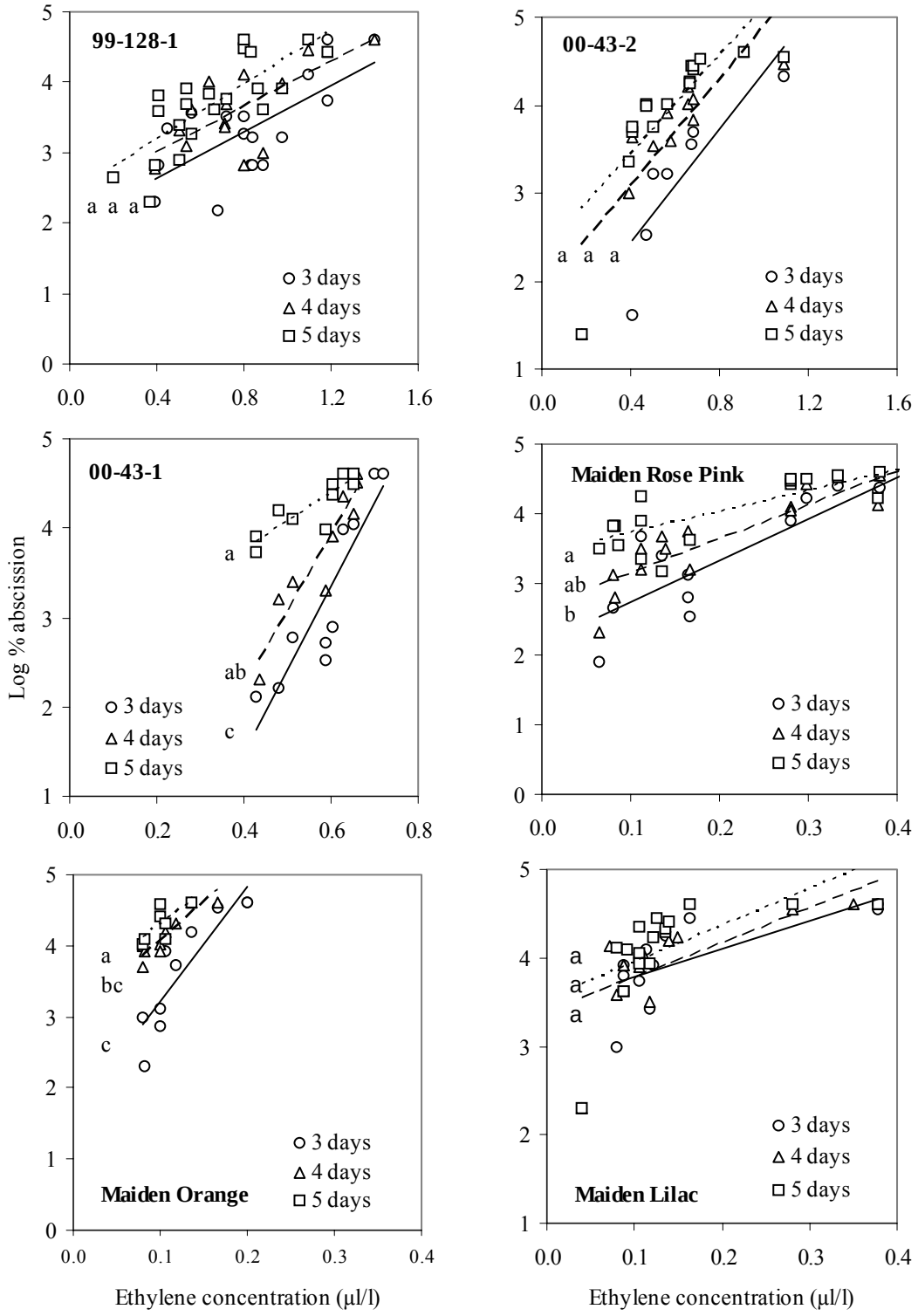


Figure 4.6-2. The effect of ethylene concentration on petal abscission for 3-, 4- and 5-day old florets, replotted from figure 4.6-1. The regression function was linearized by log transformation of % abscission. Note variation in scale of x-axis.

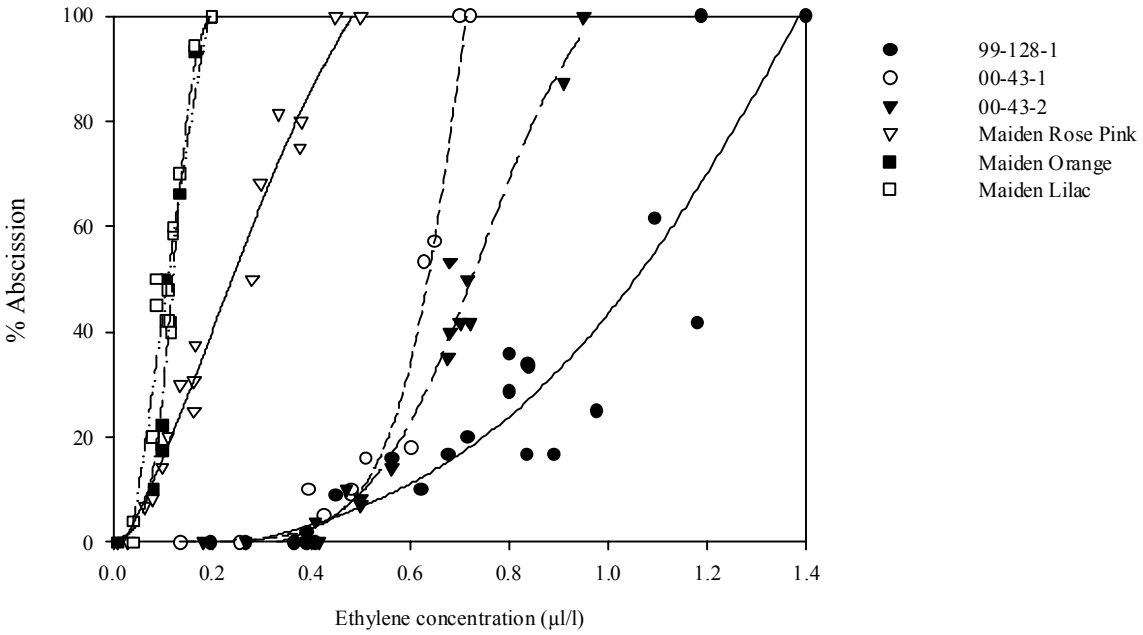


Figure 4.7. The effect of ethylene concentration on petal abscission for three-day old florets of 6 different genotypes (Season 2). Each point represents the percent abscission of at least 8 florets in a single experiment. The variables cultivar and ethylene concentration significantly affected petal abscission at $P < 0.001$.

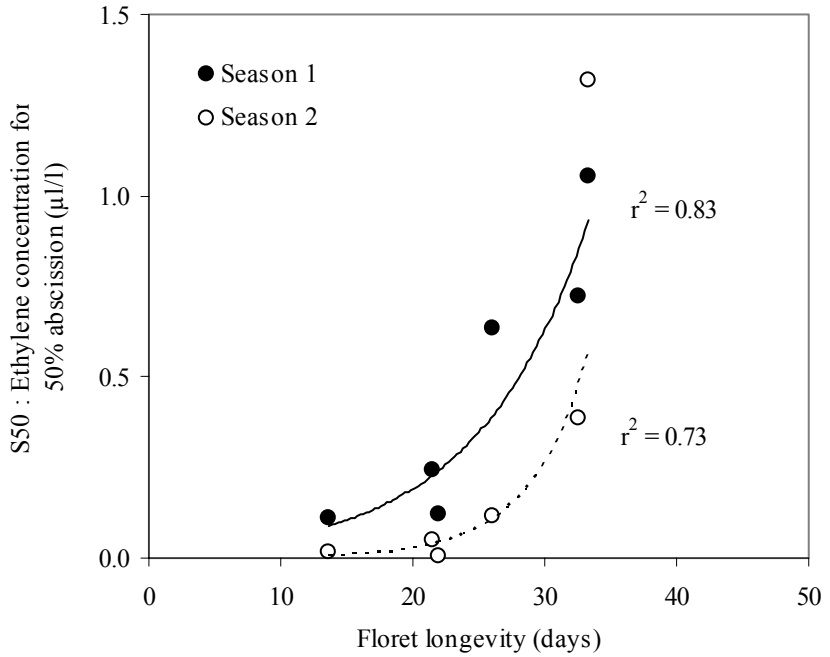


Figure 4.8. The relation between floret longevity and ethylene concentration for 50% petal abscission for 3-day old florets. All the genotypes shown in Figure 4.7 were included in this figure.

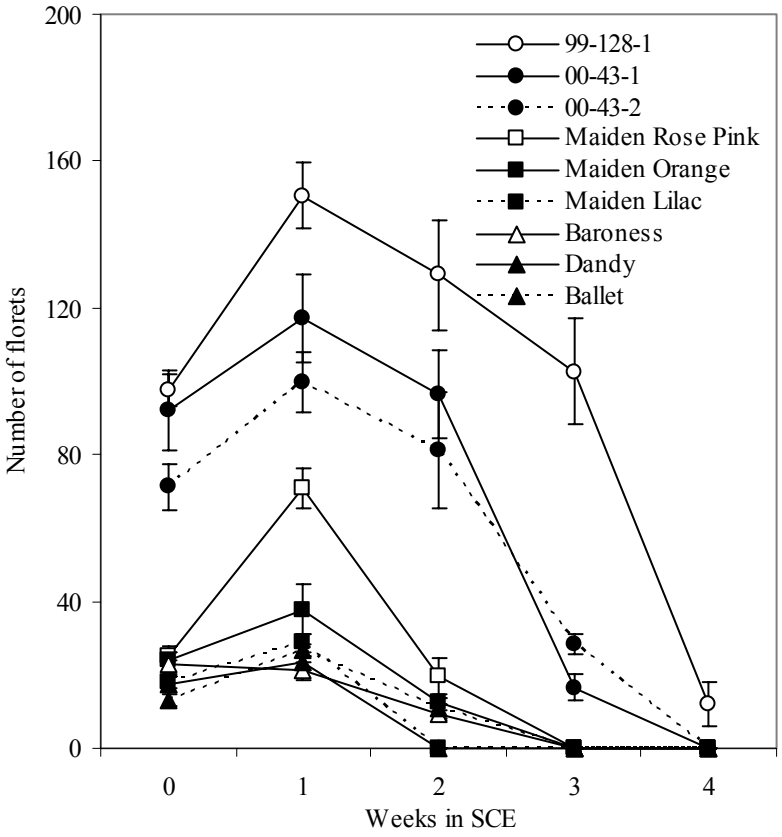


Figure 4.9. Changes in flower number produced by nine genotypes during 4 weeks in a SCE after pretreatment with 1 µl/l 1-MCP followed by 0.3 µl/l ethylene exposure. Data are shown only for control plants since there was no treatment effect. Values shown are means of number of florets for at least 4 plants ± SE.

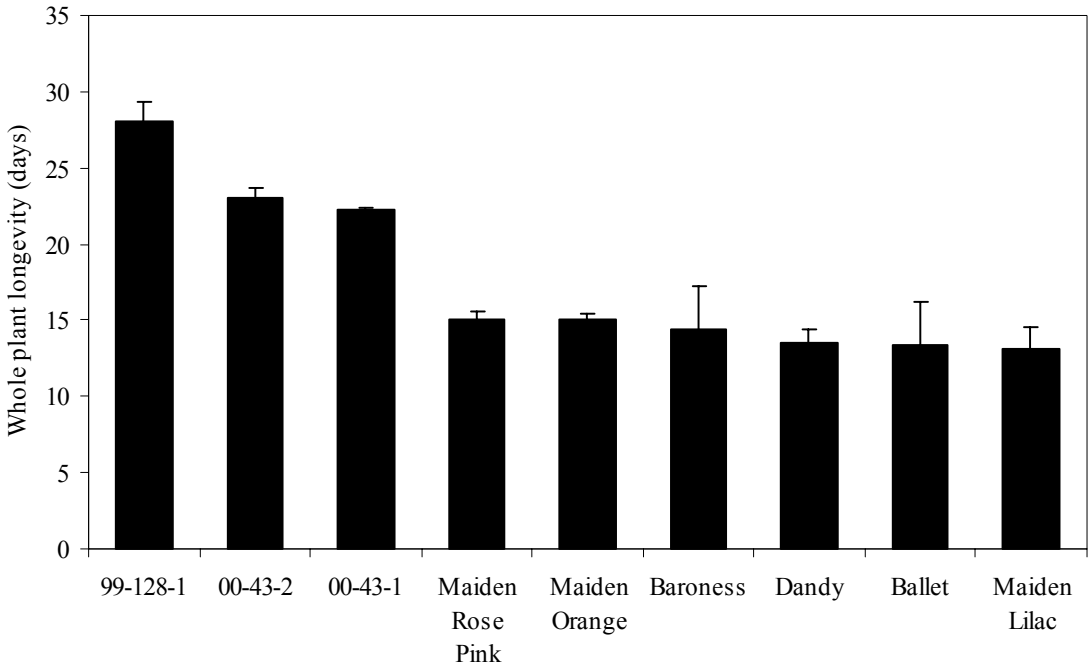


Figure 4.10. Whole plant longevity in a SCE after pretreatment with 1 µl/l 1-MCP followed by 0.3 µl/l ethylene exposure. Data shown are means of whole plant longevity for at least 4 plants ± SE.

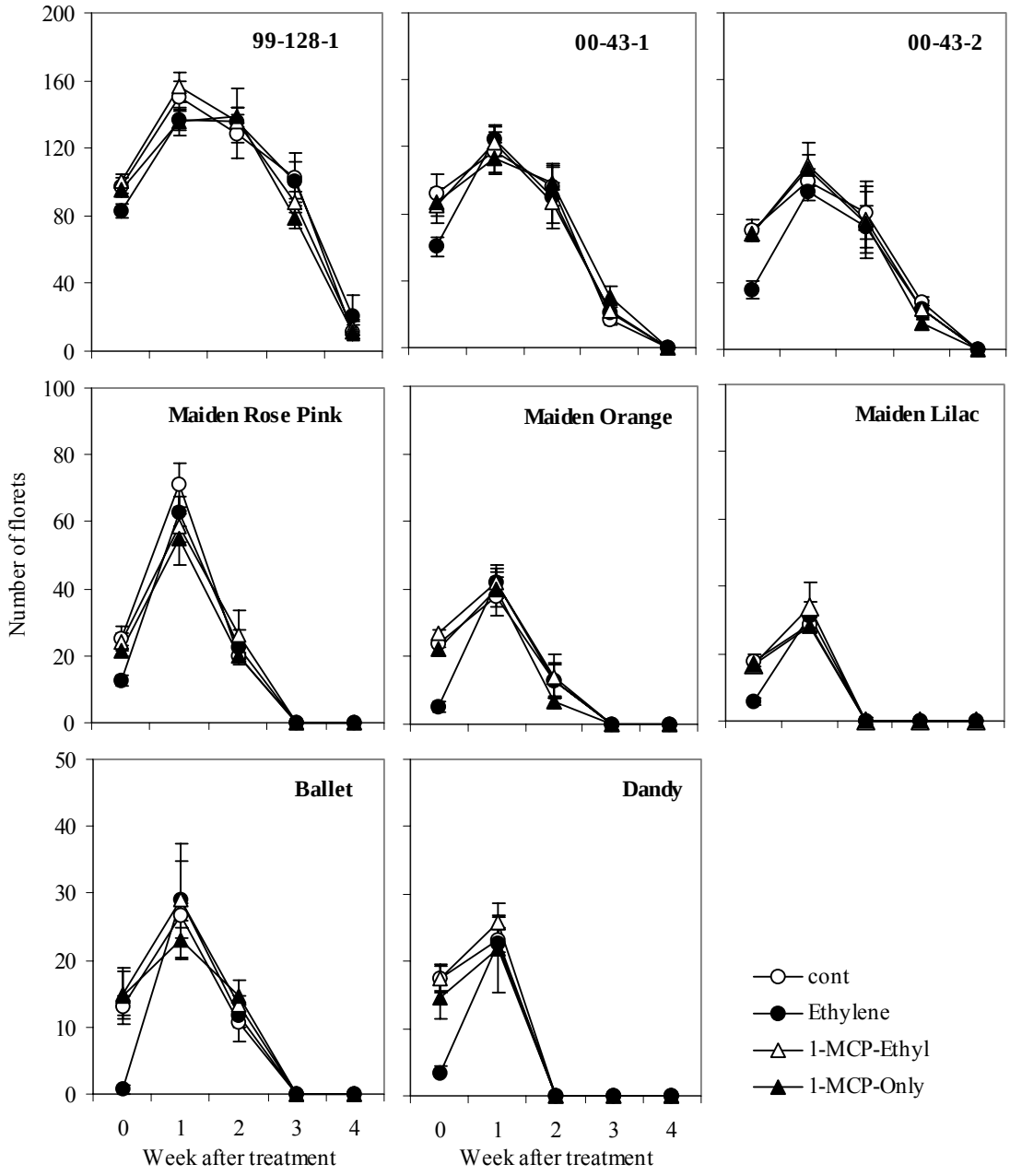


Figure 4.11. Change in the number of florets in a SCE after pretreatment with 1 µl/l 1-MCP followed by 0.3 µl/l ethylene exposure. Data shown are means of average number of florets for at least 4 plants ± SE. Note variation in scale of y-axis.

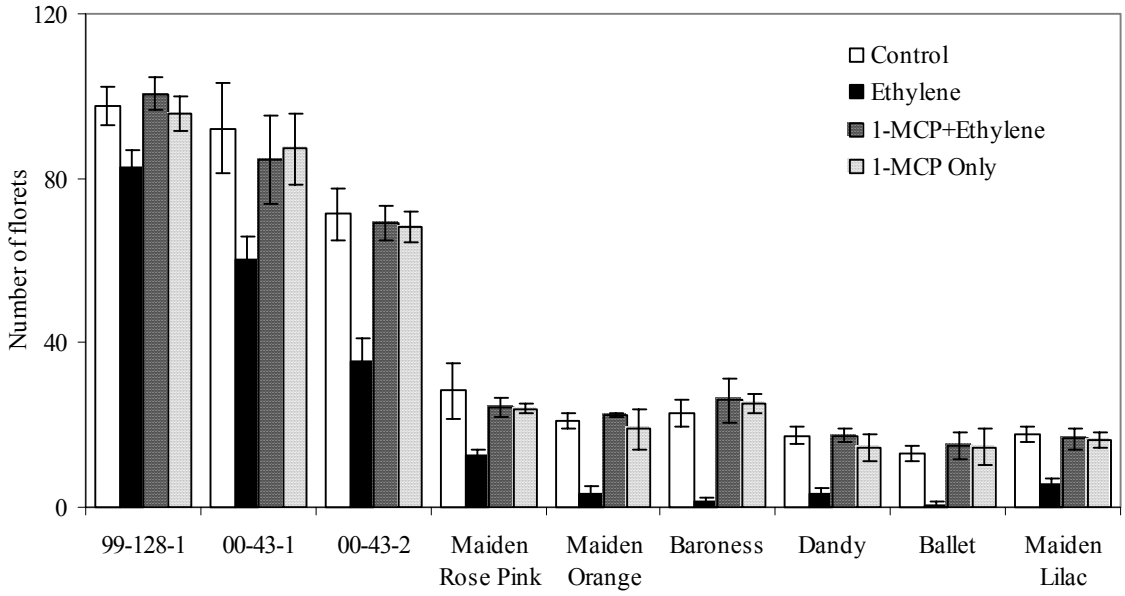


Figure 4.12. Number of florets per plant after pretreatment with 1-MCP followed by ethylene treatment. The evaluation was done immediately after ethylene treatment (Day 0). Reduction in number of florets was observed only in ethylene treatment. Data shown are means of number of florets on at least 4 plants ± SE.

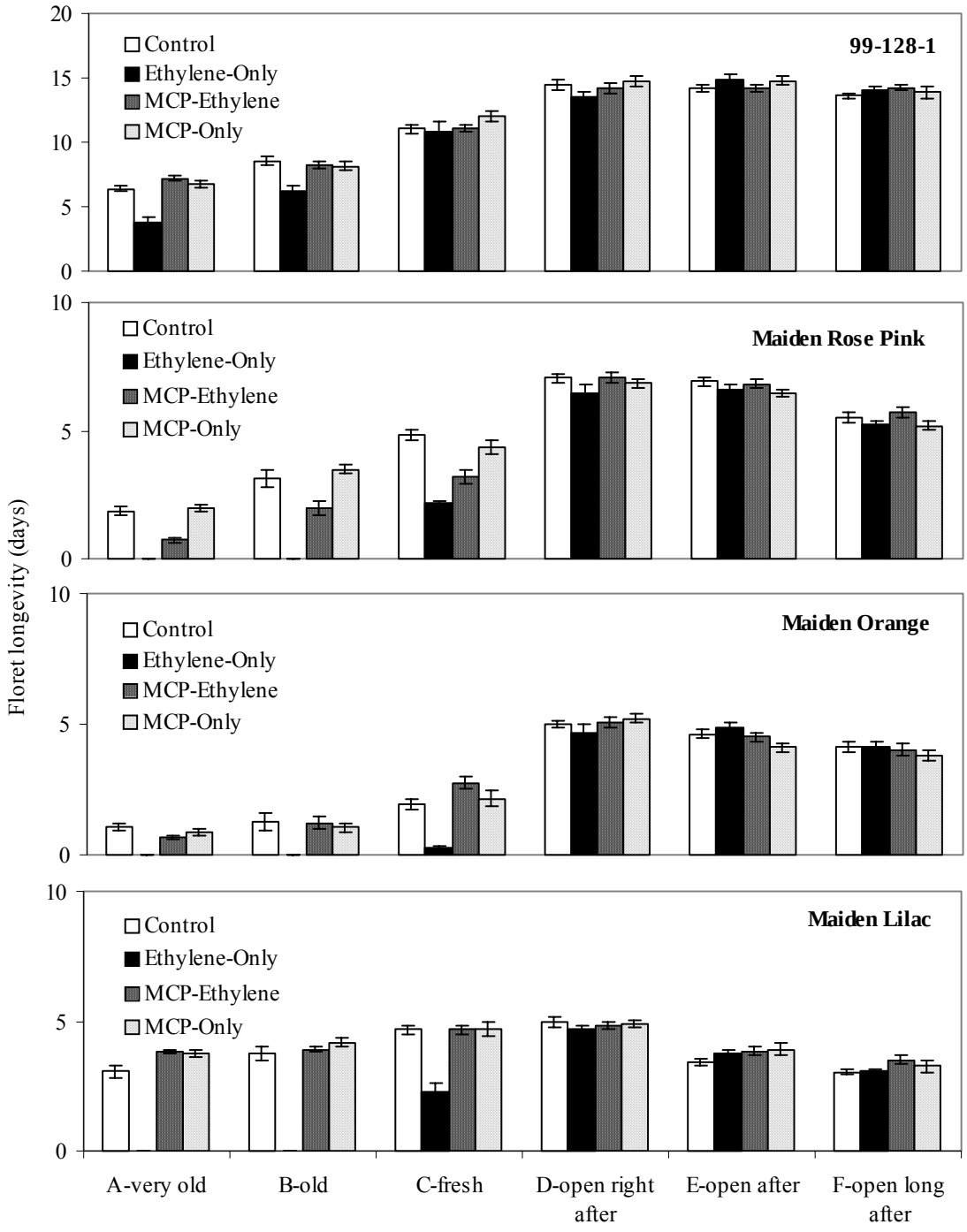


Figure 4.13. Reduction of floret longevity by ethylene in older florets. 1-MCP protected plants from the effects of ethylene but had no effect on florets opening after the 1-MCP treatment. A-very old; 5 days or more (up to 8 days), B-old; 3 to 4 day old, and C-fresh; 1 to 2 day old at the time of treatment, D-open right after; open 0 to 1 day, E-open after; open 2 to 6 day and F-open long after; 7 days or more after treatment. Data shown are means of floret longevity for at least 4 plants ± SE. Where bars appears to be missing, value was zero. Note variation in scale of y-axis.

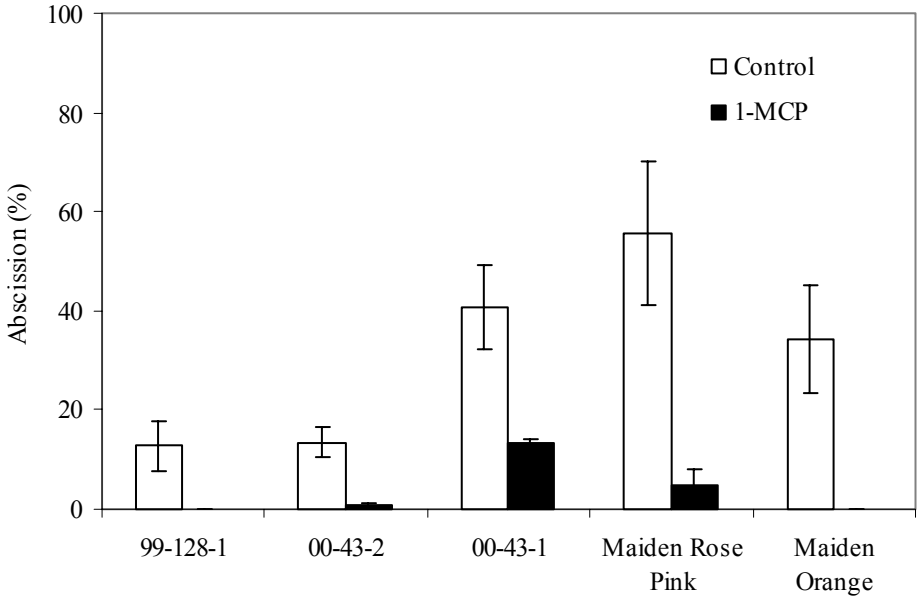


Figure 4.14. Effects of cultivar and pretreatment with 1-MCP on petal abscission during simulated transport. Data shown are means of % abscission for at least 6 plants ± SE.

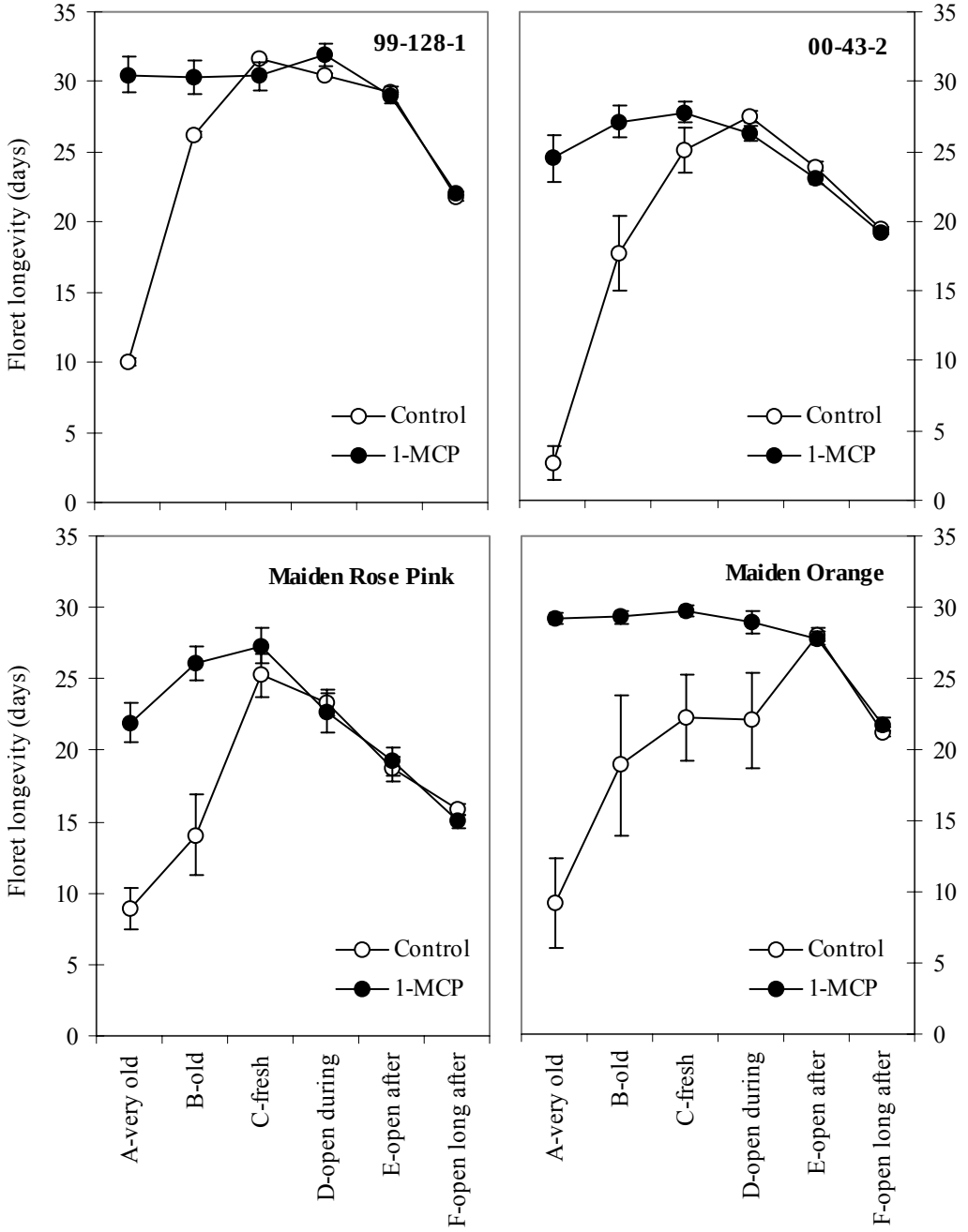


Figure 4.15. Effects of cultivar and floret age on floral longevity in the SCE after pretreatment with 1 µl/l 1-MCP for 4 h followed by simulated transport for 3 days at 5 C and 95 % RH in darkness. Ethylene detected during simulated transport was 0.067 ± 0.004 µl/l. A-very old; 5 days or more (up to 10 days), B-old; 3 to 4 day old, and C-fresh; 1 to 2 day old at the time of simulated transport, D-open during; open during simulated transport, E-open after; open 4 to 6 day and F-open long after; 7 days or more after simulated transport. Data shown are means of floret longevity for at least 6 plants ± SE.

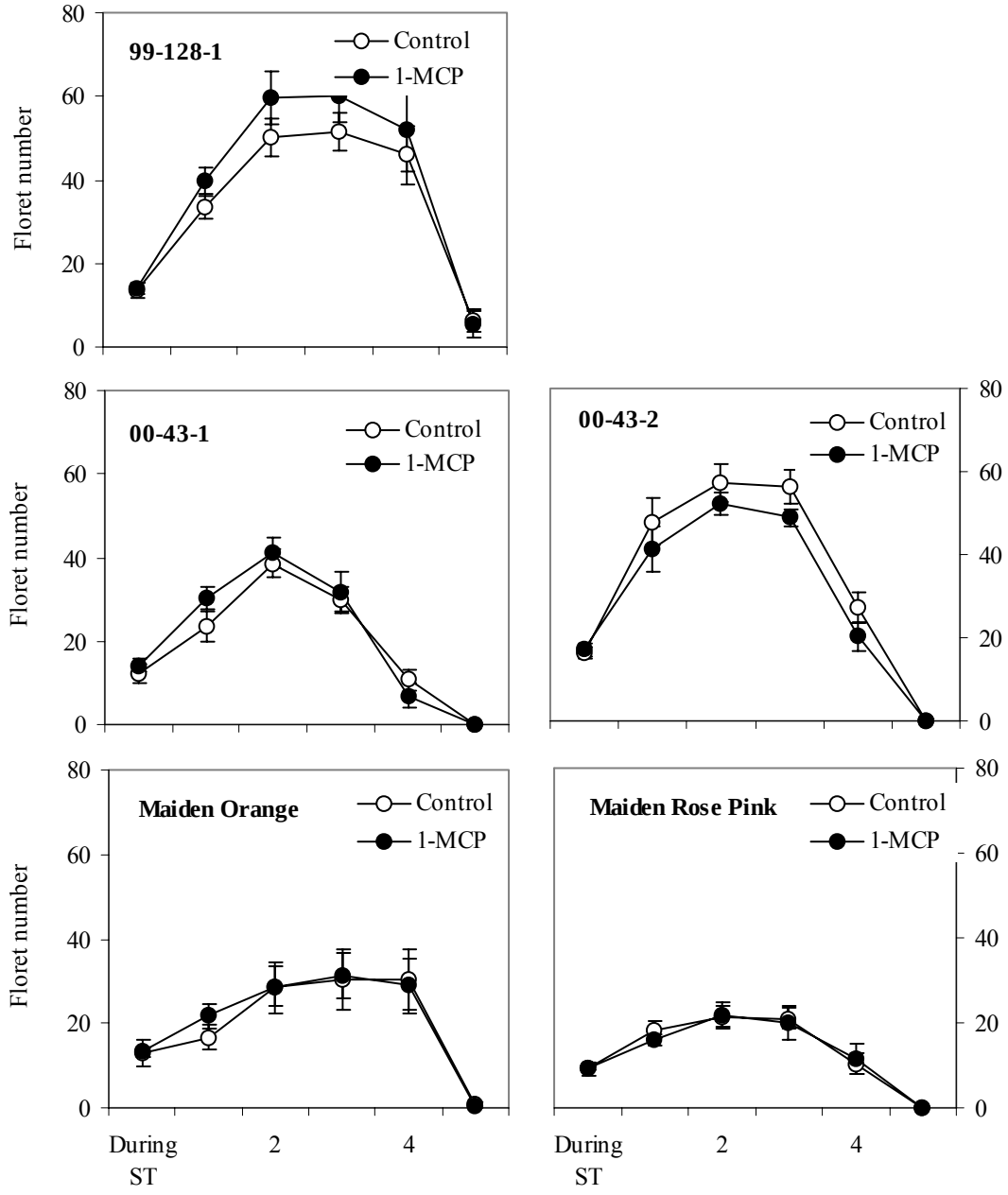


Figure 4.16. Change in the number of florets in a SCE after pretreatment with 1 µl/l 1-MCP followed by simulated transport). Data shown are means of floret number for at least 6 plants ± SE.

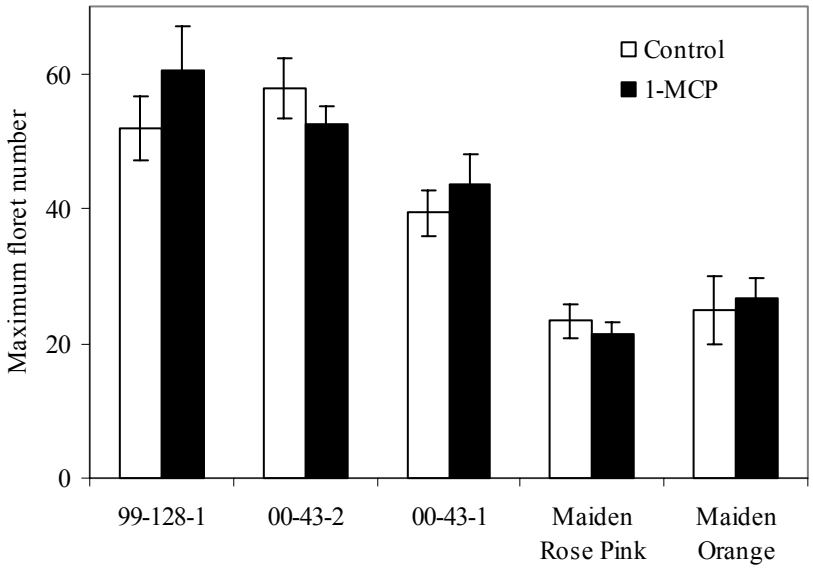


Figure 4.17. Effects of 1-MCP pretreatment on maximum floret number in the SCE after simulated transport for 3 days at 5 C and 95 % RH in darkness. Data shown are means of maximum floret number for at least 6 plants ± SE.

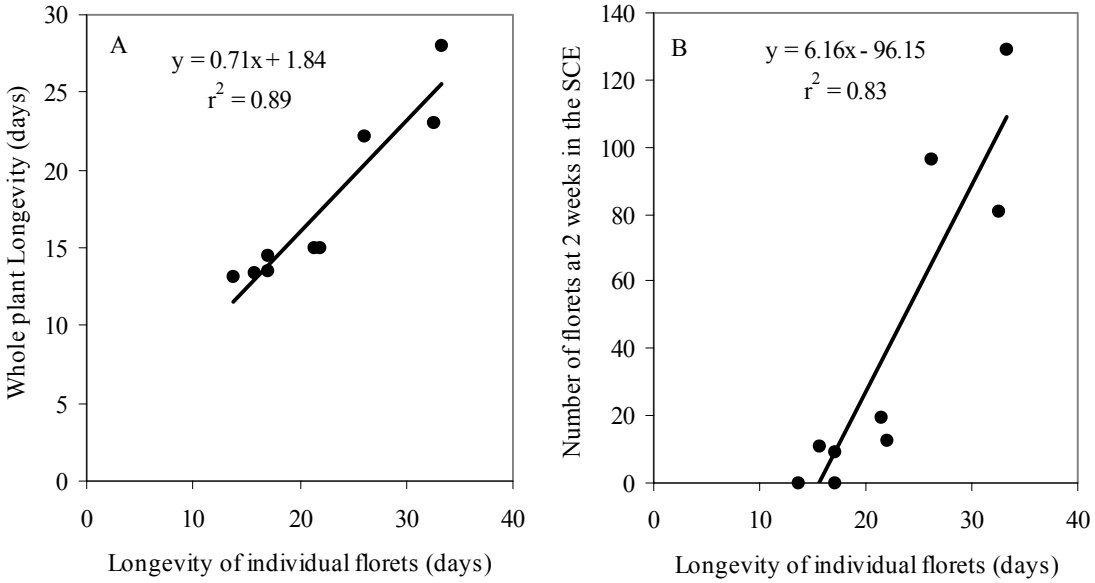


Figure 4.18. Correlation between (A) longevity of individual florets on intact plants and whole plant longevity in the SCE, (B) longevity of individual florets on intact plants and floret number of whole plants at 2 weeks in the SCE. All the genotypes shown in Figure 4.12 were included in this figure.

# Appendix

**Degree of white fly infestation in Regal Pelargoniums**

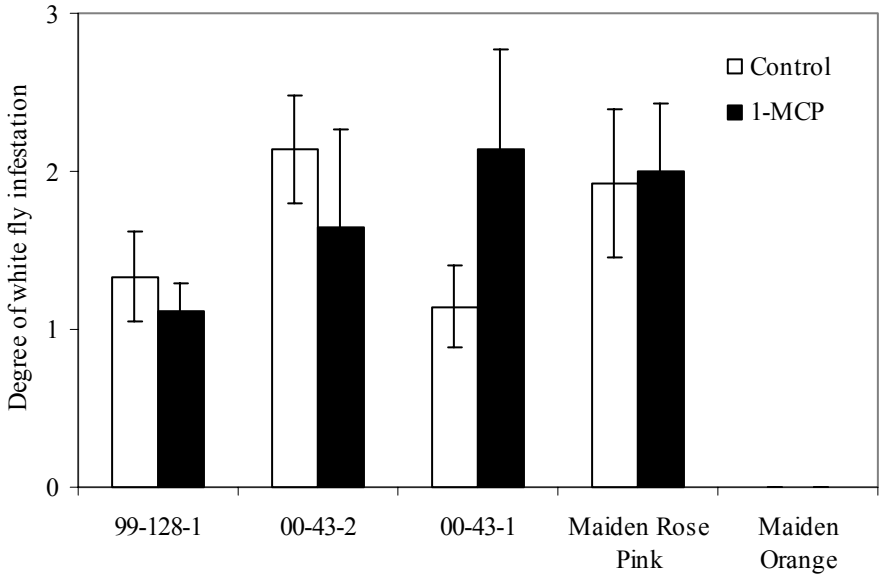


Figure1. Effects of cultivar on degree of white fly infestation in the SCE after pretreatment with 1-MCP. The qualitative assessment of their population levels was carried out based on a scale of 0=no leaves infested, 1=1-25, 2=26-50, 3=51-75, 4=76-90, and 5=90≤% leaves infested. Data shown are means of degree of white fly infestation for at least 6 plants ± SE.

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### Publications

Brown, KB YJ Zhang, HJ Kim, and JP Lynch. 2004. The ethylene underground. Proceedings of the XXVI International Horticultural Congress: Environmental Stress and Horticulture Crops. Acta Hort. 618: (no page numbers yet).
Kim HJ, K.Ohkawa, Y Kuda, and JN Suh. 1998. Effect of combined pretreatment and stem recutting on cut rose flowers after postharvest pretreatment and simulated transport. Suppl. J. Japan. Soc. Hort. Sci. 67 (1) '98, 287.
Suh, JN, HJ Kim, Y Kuda, and K Ohkawa. 1997. Effect of stem recutting on fresh weight and vase life of cut rose flowers after postharvest pretreatment and simulated transport. Suppl. J. Japan. Soc. Hort. Sci. 66 (2) '97, 66-67.
Suh, JN, Y Kuda, HJ Kim, and K Ohkawa. 1997. Cultivar difference in senescence symptoms of *Cymbidium* flowers after emasculation. Suppl. J. Japan. Soc. Hort. Sci. 66 (2) '97, 64-65.