PHYTOTRON REPORT
2005

NC STATE UNIVERSITY
# PHYTOTRON STAFF

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
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<tbody>
<tr>
<td>Director</td>
<td>Judith F. Thomas</td>
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<tr>
<td>Director Emeritus</td>
<td>Robert J. Downs</td>
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<tr>
<td>Assistant Director</td>
<td>Carole H. Saravitz</td>
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<tr>
<td>Administrative Secretary</td>
<td>Judy R. Edwards</td>
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<tr>
<td>Research Unit Manager</td>
<td>Janet L. Shurtleff</td>
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<tr>
<td>Research Mechanics</td>
<td>Walter E. Diamond</td>
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<tr>
<td></td>
<td>Sean E. Hussey</td>
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<tr>
<td>Electronics Specialists</td>
<td>Richard C. Evans</td>
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<td>Thomas M. Winkler</td>
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<td>Phytotron Manager</td>
<td>Charles M. Gibbs</td>
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<td>Phytotron Technicians</td>
<td>James W. Cojocari</td>
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<td>Robert E. Jones</td>
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<tr>
<td>Phytotron Assistants (Part-Time Students)</td>
<td>Anthony Alexander II</td>
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<tr>
<td></td>
<td>Aaron Caldwell</td>
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<td></td>
<td>Terri Eller</td>
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<td>Zane Gooding</td>
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<td>Richard Jankovics</td>
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<td></td>
<td>Ian Justus</td>
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<td>Ana Pardo</td>
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<td>Daniel Peters</td>
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<td>Anthony Sanders</td>
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<td>Tony Scott</td>
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<td>Rose Somody</td>
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<td>Jonathan Zehr</td>
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Cover: Geranium (*Pelargonium X hortorum* Bailey)
NCSU Phytotron
A Controlled Environment Facility
Annual Report 2005

North Carolina State University

College of Agriculture and Life Sciences
Tomorrow’s Science And Technology...Today
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Usage by Chamber

Usage for all growth chambers in 2005 was 95% of the recommended optimal occupancy\(^1\), or 78% of maximal occupancy (Table 1). The 17 individually programmed A-chambers were occupied at 136% of optimal capacity and the five “standard”\(^2\) A-chambers had a 63% optimal occupancy rate; total A-chamber usage was 120% for 2005. Usage of B-chambers was at 82% and C-chambers, 84% for the year.

During 2005 the glasshouses had an optimal occupancy rate of 44%. A large portion of the glasshouse space allocation was utilized for studies with student projects (Bio 183), corn (Allen, Botany; Davis, Botany; Holland, Crop Science; Balint-Kurti, Plant Pathology), turfgrass (Dong & Qu, Crop Science), cotton (Haigler, Crop Science), tomatoes Sederoff & Khodakovskaya), wheat (Qu, Crop Science) and to study the hydraulic architecture of redbay trees (Hoffman, Botany).

The two walk-in rooms equipped with high intensity discharge lamps (HID) were in use during 92% of the year for studies examining the growth responses of soybean (Israel, Soil Science; Burton & Villagarcia, Crop Science). Several studies examining chilling injury were conducted in the two B chambers that can be set to low temperatures. Cold temperature studies included: chilling injury in cucumber and watermelon seedlings (Kozik & Wehner, Horticultural Science), strawberries (Poling, Horticultural Science) and Argentine ants (Silverman, Entomology).

Usage by Department

70 different projects were conducted in the Phytotron during 2005 by faculty and students from 9 departments in the Colleges of Agriculture and Life Sciences (Table 2). The list of projects conducted in the Phytotron during 2005 follows the reports. The Crop Science Department used the largest amount of space in 2005, nearly 35%, for 21 different projects. Secondly, the Plant Pathology Department used more than 12% of the space for 13 projects. The Botany Department used nearly 9% of the space for 15 projects, and Horticultural Science used over 8% for 7 projects. Genetics, and Soil Science each had
space use allocations of approximately 5-7%. The Department of Entomology used almost 3% and approximately two percent of the space was used by Biological Sciences during the BIO 183 class for student projects.

**Usage by Crop Type**

17% of the space used in the Phytotron during 2005 was used to grow corn while 15% was allocated for growing arabidopsis (Table 3). Looking at footnote “c” it is apparent that many studies were conducted with non-crop type species, including many different weeds, as well as Argentine ants, Honey bees, *Cissus trifoliata, Anthurium crentum, Casearia guianensis, Lotus japonicus, Medicago truncatula*. Nearly 27% of the space was allocated to projects using tobacco which included continued research on the blue mold disease problem, research with transgenic tobacco, and studies with different species of tobacco: *N. benthamiana* and *thrysiflora*. Research with field crops included soybeans (11%),) and cotton (32%). Space for research on vegetable crops used 9% of the space in 2005, strawberries, 1% of the space, ornamentals, 5% and for trees, 3%. The ‘Demonstration’ category (2%) included space for plants grown for display during tours, and for the BIO 183 research projects.

**Phytotron Visitors**

A portion of our outreach and educational goals includes offering tours of the Phytotron. During the year laboratory sections from NCSU general botany, crop science, and horticultural science classes; Meredith College; and numerous high school and junior high biology classes and biology clubs visited the facility. Students from 4-H, Future Farmers of America and various science camps have also toured the Phytotron. Visitors also came from China and Mexico.

Our tours usually consist of a walk through the building with various stops at projects of special interest such as the air quality exposure chambers, the hydroponics units, new high intensity light chambers, glasshouses, and plant pathology and zoology projects. The tours generally are an hour long and include a demonstration of plant response to short-day and long-day photoperiods over a range of temperature regimes. On an average we host at least one group of visitors (ranging in size from 1 to 50) per week.
who desire to learn about the various biological and engineering aspects of controlled environment research. Groups larger than 10-12 people require that simultaneous tours must be provided by the Director, Assistant Director, and Research Unit Manager. Tours must be booked in advance through the Administrative Secretary. In addition to providing educational tours, we also provide plant materials to demonstrate the effects of temperature and photoperiod on plant growth and development for classes in Departments of Botany and Crop Science. We also worked with 230 students from August – December and 40 students from January – March from the BIO-183 Introductory Biology class. We provided them a 'hands-on' experience in environment and experimental control of plant growth.

**Phytotron Projects**

Special projects in the Phytotron during 2005 included modification of the Phytotron Space Use Program. The new space use program integrates the space use data with the phytotron proposal forms that are submitted to request chamber space. The integration of the 2 programs makes it easier for phytotron users to renew their space and has streamlined the collection of data concerning space use.

**General Usage Information**

Phytotron space use rental fees applicable to grant-supported research and to off-campus users is currently $1.47 per truck (unit) per day. The fee for an individual A-chamber is $36.00/day; for a B-chamber, $12.00/day; for a C-chamber, $4.50/day; and the $1.47/truck/day applies to space occupied in either the "standard" chambers or in the glasshouses. Fees include usage of plastic pots and substrate mixes, Phytotron nutrient solution and deionized water, and certain equipment such as balances, leaf area meter, drying oven, etc. Employment of part-time assistance for off-campus users can be arranged through the Director. Space use request forms are available on our website at http://www.ncsu.edu/phytotron/application.html.

1 Usage calculations for A-chambers assume that the chambers contain a maximum of 24 units or 'trucks'. Optimal occupancy is set at 15 units, however, in order for there to be
space for the investigator to work, for the staff to water plants and change lamps and wall fans, and to prevent overcrowding and shading of experimental material. B- and C-chambers usage is calculated on the basis of maximum occupancy since their small sizes allow for reach-in care by investigators and staff.

2 Standard A-chambers are set at 4 day/night temperature regimes of 26/22, 22/18, and 18/14 C. There are 2 chambers for each temperature regime, both programmed for a 9-hr high intensity light period coincident with the day temperature; one of the two chambers has a 15-hr dark period following the high intensity light period (simulating a short-day photoperiod) and the other chamber has a 3-hr low intensity light interruption provided by the incandescent lamps during the middle of the dark period (simulating a long-day photoperiod).
Table 1. **CHAMBER USAGE SUMMARY, 2005**

<table>
<thead>
<tr>
<th>Chamber*</th>
<th>% Optimal</th>
<th>% Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-chambers (17 individual)</td>
<td>136</td>
<td>85</td>
</tr>
<tr>
<td>A-chambers (5 standard)</td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td>A-chambers (22)</td>
<td>120</td>
<td>71</td>
</tr>
<tr>
<td>B-chambers (10)</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>C-chambers (22)</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Glasshouses (5)</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>HID Walk-in (2)</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Tall Chamber (1)</td>
<td>43</td>
<td>40</td>
</tr>
</tbody>
</table>

* Dimensions of Chambers are: Utilization of all growth chambers during 2005:

A = 8' x 12' x 7'h  
Optimal Usage = 95

B = 8' x 4' x 7'h  
% Maximal Usage = 78

C = 4' x 3' x 4'h

H = 10' x 6' x 8'h

T = 16' x 12' x 7'-15'h

Table 2. **DEPARTMENT USAGE SUMMARY, 2005**

<table>
<thead>
<tr>
<th>Department</th>
<th>% Total Use-Days</th>
<th># Projects</th>
</tr>
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<tbody>
<tr>
<td>Botany</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Crop Science</td>
<td>35</td>
<td>21</td>
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<tr>
<td>Entomology</td>
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<td>4</td>
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<tr>
<td>Genetics</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Horticultural Science</td>
<td>8</td>
<td>7</td>
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<tr>
<td>Phytotron</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Plant Pathology</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Soil Science</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Teaching</td>
<td>2</td>
<td>2</td>
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</table>

*70 Studies Conducted in the Phytotron During 2005
### Table 3. CROP TYPE SUMMARY, 2005

<table>
<thead>
<tr>
<th>Crop</th>
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<tbody>
<tr>
<td>Arabidopsis</td>
<td>15</td>
</tr>
<tr>
<td>Corn</td>
<td>17</td>
</tr>
<tr>
<td>Cotton</td>
<td>2</td>
</tr>
<tr>
<td>Demonstration&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>Ornamentals&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>Other&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>Peanut</td>
<td>1</td>
</tr>
<tr>
<td>Rice</td>
<td>3</td>
</tr>
<tr>
<td>Soybean</td>
<td>11</td>
</tr>
<tr>
<td>Teaching</td>
<td>1</td>
</tr>
<tr>
<td>Tobacco</td>
<td>7</td>
</tr>
<tr>
<td>Trees&lt;sup&gt;d&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Turfgrass&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td>Vegetables&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9</td>
</tr>
</tbody>
</table>

Includes:

<sup>a</sup>Celosia, Corn, Himalayan Barley, Marigolds, Mung Beans, Peas, Pigweed,
<sup>b</sup>Dogwood, Geranium, Helleborus, *Setcreasia purpurea*, Rhododendron
<sup>d</sup>Fraser Fir, *Fraxinus americana & pennsylvanica*, *Nyssa sylvatica & aquatica*, Redbay, *Vaccinium corymbosum & stamiana*, *Viburnum dentatum & rudifilum*
<sup>e</sup>Bentgrass (*Agrostis palustris*), Bermudagrass, Saint Augustinegrass, and Tall Fescue
<sup>f</sup>Cucumber, Potato, Tomato
Introduction

Current ground-level O₃ concentrations suppress biomass and yield in both agronomically-important species and natural vegetation in many regions worldwide. Ambient O₃ suppresses yields of susceptible crops such as soybean, cotton, peanut, rice, and wheat by 5 to 15% annually (Heagle, 1989). Current losses to US agriculture are substantial: a 25% reduction in ambient O₃ would benefit US agriculture by $5.2 billion annually (2005 dollars) (Adams and Horst, 2003). With projected increases in tropospheric O₃ concentrations (Prather et al., 2003), improved plant tolerance to O₃ is vital to meeting future global food and biomass demands.

However, the etiology of O₃ toxicity remains unclear. Reactive oxygen species (ROS) derived from reactions between O₃ and cellular components appear to inflict biochemical lesions and propagate defense responses that compound plant injury (Fiscus et al., 2005). ROS may act as signaling molecules to induce certain antioxidative stress-related defense responses (Long and Naidu, 2002). Because some plant defense responses involve membrane-associated GTPases (G proteins), we are evaluating the O₃-sensitivity of Arabidopsis thaliana mutants altered in the heterotrimeric G-protein pathway (Booker et al., 2004). The heterotrimeric G-protein conveys signals from the extracellular space to cytosolic downstream effectors (Jones, 2002). Possibly, eventual effectors include peroxidases. Following O₃ exposure, the activities of leaf peroxidases increase (Booker et al., 1992; Sharma and Davis, 1997; Ranieri et al., 2000; Ranieri et al., 2003; Booker and Fiscus, 2005). We used a collection of signal-transduction knockout mutants to examine
regulation of peroxidases following low-level O₃ exposure. The effect of O₃ on peroxidase activity in known O₃-sensitive and -resistant mutants was followed as well.

Materials and Methods

Twelve genotypes were treated with either charcoal-filtered (CF) air or CF-air plus 125 nmol O₃ mol⁻¹ 8 h daily for 2 days in four continuous-stirred tank reactors (CSTRs) operating in chamber A-6 in the NC State University Phytotron (Fig. 1). The O₃ treatment level was about 2.5 x daily average ambient concentrations measured in our area during the summertime. The CSTRs are designed for the rapid mixing of gases, and the A-6 chamber provides controlled temperature, relative humidity and light conditions.

The facility was upgraded again this year. We installed computer-controlled O₃ dispensing and monitoring equipment that provides for continuous feedback control of O₃ treatment concentrations. This allows for user-defined O₃ exposure profiles and remote monitoring of system status. Environmental data acquisition capability was also enhanced and now includes CO₂ monitoring. This is now a state-of-the-art facility.

A spectrophotometric assay was used to examine whole-leaf peroxidase activity. The distal portion of four leaves per plant was sampled immediately following the two-day O₃ treatment. Tissues were ground in 0.6 ml of 50 mM KPi buffer (pH 6.8) and 25 mg of PVPP/acid-washed quartz sand in a glass homogenizer. Plant extracts were centrifuged at 21,000 x g for 8 min at 4 °C. The supernatants were recovered and 50-ul aliquots were assayed for peroxidase activity in a 2 ml volume containing 50 mM Na acetate buffer (pH 5.0), 0.5 mM H₂O₂ and 0.5 mM of an artificial electron donor. The experiment was conducted three times with two replicate chambers per treatment on each occasion.

Results and Conclusions

Following as little as 8 hrs of exposure to a physiologically-relevant O₃ concentration (125 ppb), measurable differences in peroxidase activity were detected in
this generally O₃-tolerant plant species. This is a unique discovery since the leaves do not exhibit any O₃-related symptoms (i.e., leaf curling) or chlorosis/necrosis. The use of environmentally-relevant concentrations improves the applicability of findings to field conditions. Further, the use of lower O₃ levels than are typically used in genetic screens with Arabidopsis improves the likelihood that we measured signal transduction events in plants, rather than massive insult by a potent oxidant.

The screening of the 12 mutants for O₃-induced peroxidase activity produced some surprising responses. Our previous work (Booker et al., 2004) found phenotypic differences between the β subunit of G-protein (agb1-2) and α subunit of G-protein (gpa1-4) mutant responses to elevated O₃. However, there was not a difference in O₃-induced alteration of peroxidase activity among these signaling mutants. Their responses were similar to the wild-type plants (Fig. 2). The increase in peroxidase activity in the radical-induced cell death 1 (rcd1) and ethylene-overproducing (eto1) mutants was consistent with their high O₃ sensitivity, while in contrast the known O₃-sensitive jasmonic acid resistant (jar1) mutant did not exhibit a change in peroxidase activity. The ethylene-insensitive (ein2), O₃-tolerant mutant had a high constitutive peroxidase activity, but it was not stimulated further by O₃. The G-protein coupled receptor (gcr; plasma membrane) likewise had an unaltered response relative to wild-type, but other membrane-bound receptors (mutation-induced recessive alleles; mlo; mildew resistant) had differing O₃ responses.

These results suggest that multiple signaling pathways are operating in O₃-exposed plants. Pathways involving ethylene appear linked to peroxidase responses while pathways involving jasmonic acid-mediated sensitivity operate independently of peroxidase. A recent study suggests that increased susceptibility of Gβ-deficient mutants to necrotrophic pathogens was related to decreased sensitivity to jasmonic acid (Trusov et al., 2006). This provides a clue as to why increased O₃ sensitivity of agb1-2 was not reflected by higher peroxidase activities. Toxicity may be inflicted through a jasmonic acid-related pathway that does not involve peroxidase. Studies are in progress to better define these putative multiple signaling pathways.
Fig. 1. Treatment of *Arabidopsis* genotypes with O$_3$ was conducted in four continuous-stirred tank reactors (CSTRs) in an A-chamber in the NC State University Phytotron. The CSTRs provide rapid mixing of charcoal-filtered air with prescribed levels of O$_3$ at controlled temperature, relative humidity and light conditions. Facility upgrades installed this year provide for user-defined O$_3$ exposure profiles, computer-controlled continuous-feedback O$_3$ dispensing and monitoring capabilities, remote monitoring, and enhanced environmental data acquisition, including CO$_2$ monitoring. This is a state-of-the-art facility.
Fig. 2. Peroxidase activity in whole-leaf extracts of *Arabidopsis* mutants (Columbia background) following O$_3$ exposure. Plants were exposed to charcoal-filtered air (CF) or CF air plus 125 nmol O$_3$ mol$^{-1}$ 8 h daily for two days. Genotypes were Columbia-O (Col), null β subunit of G-protein (*agb1-2*), null α subunit of G-protein (*gpa1-4*), double null G-protein (*agb1-2/gpa1-4*), null G-protein coupled receptor (*gcr*), accumulation and replication of chloroplasts (*arc*), radical-induced cell death 1 (*rcd1*), ethylene insensitive 2 (*ein2*), ethylene overproducing (*eto1*), jasmonic acid resistant (*jar1*), and mutation-induced recessive alleles (*mlo*, mildew resistant). Total peroxidase activity was assayed in three replicate experiments with two replicate chambers per treatment on each occasion ($n = 6$). Statistically significant differences between CF and O$_3$ treatments within each genotype are denoted with $0.05 < P < 0.1$ (bracketed *); $0.01 < P < 0.05$ (*); $0.001 < P < 0.01$ (**); and $P < 0.001$ (***)
References


ECOLOGICAL AND PHYSIOLOGICAL BASIS FOR THE DISTRIBUTION OF WOODY PLANTS ALONG WATER AVAILABILITY GRADIENTS IN THE EASTERN UNITED STATES MIXED FOREST

Pamela P. Abit and William A. Hoffmann

Introduction

Broad-leaved deciduous trees dominate mixed forest in the Eastern United States (US). In the Koppen-Trewartha system of climate classification, Eastern US lies within the Cf climate, which is described as temperate and rainy with hot summer. The Cf climate has no dry season and receives at least 30 mm (1.2 in.) of rain in the driest summer months (USDA Forest service). In addition, precipitation may even reach 60 inches per year in the Southeastern part (CA, Dept. of Forest UI-Urbana, 1974). Under such climates, it is expected that water is not a limiting factor in the deciduous forest biome of the eastern US.

Nevertheless, the distribution of species in eastern forests suggests that water availability does exert a strong control on the success of woody plants. Xeric forest on dry sites is typically dominated by drought-tolerant species like the white, red and black oak canopies. On the other hand, mesic forest on sites with moderate soil moisture is dominated by stable maple basswood forest. Some species are observed to survive in a wide range of environmental conditions with different water availability regimes while others are confined to a particular habitat. According to Sellin (2001), occurrence of woody plant species is largely controlled by water availability as this can affect growth and distribution of plants or natural vegetation by affecting their physiological processes and conditions. Thus, water availability is one of the most important factors that determine the distribution of plants. The distribution of these woody plant species along water availability gradient may be influenced by their physiological modifications that are closely adjusted to the environmental conditions since terrestrial plants are exposed to fluctuations of water regimes (Barbour et al., 1999).

Although Eastern US forests are not considered to be water-limited ecosystems, water relations and other physiological filters associated with water availability that determine species composition of vegetation in a particular site such as nutrient availability, seed germination, seedling establishment and disturbance must certainly play an important role as evidenced by the distribution of species along gradients of water availability.

The study done in the phytotron focused on two of the physiological filters which include seed germination and early seedling growth and survival. We compared the ability of mesic and xeric tree species to germinate, grow and survive under varying levels of drought stress.
Materials and Method

Study species

Congeneric species pairs (genera which contain both xeric species and mesic species) of *Fraxinus* and *Nyssa* were used. Congeneric species pairs were used for ensuring phylogenetic independence, which is an important consideration for making inferences in comparative studies and in improving the statistical power of comparison between two groups (Ackerly, 1999). The species pairs used are adapted to xeric ad mesic conditions, respectively.

Germination experiment

This experiment was performed to compare the ability of mesic and xeric species to germinate under water stress. Prior to germination, the seeds of *Fraxinus americana*, *F. pennsylvanica*, *Nyssa sylvatica* and *N. aquatica* were stratified to break their dormancy. The seeds were placed on pre-soaked blotter in Petri dishes. The stratification requirements are as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Warm Period Temp, °C</th>
<th>Warm Period Days</th>
<th>Cold Period Temp, °C</th>
<th>Cold Period Days</th>
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<tbody>
<tr>
<td><em>F. americana</em></td>
<td>30/20</td>
<td>30</td>
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<tr>
<td><em>N. aquatica</em></td>
<td></td>
<td>5</td>
<td></td>
<td>30-90</td>
</tr>
</tbody>
</table>

The seeds were then surfaced sterilized with sodium hypochlorite and rinsed thoroughly with sterile distilled water. The seeds were placed on blotters in petri dishes and moistened with Polyethylene Glycol (PEG) solutions of different concentrations to simulate various water potentials as follows:

- Treatment 0- Control (deionized water)
- Treatment 1- 0.2 MPa
- Treatment 2- 0.4 MPa
- Treatment 3- 0.6 MPa
- Treatment 4- 0.8 MPa

Ten replicates of ten seeds were used for each treatment. The Petri dishes containing the seeds were placed in a growth chamber and maintained at 30 °C (day) and 20 °C (night) with 8 hours daylight and 16 hours of dark period.

The number of germinated seeds was recorded daily until no further germination occurred. Root elongation was also measured until the seedlings were seven days old.
Dry-down experiment

This experiment was performed to compare the ability of mesic and xeric species to survive extreme water stress, by evaluating survival after re-watering subsequent to drought exposure.

When *Fraxinus* seedlings from the germination study had four to six leaves, watering was interrupted until plants exhibited various wilt stages. Leaves of unstressed and stressed plants at various wilt stages were harvested and leaf water potential was measured. Subsequently, the plants were re-watered after drought exposure. Percent survival was then assessed. Figure 1 shows the different wilt stages.

*Fraxinus americana*

![Fraxinus americana Wilt Stages](image1)

*Fraxinus pennsylvanica*

![Fraxinus pennsylvanica Wilt Stages](image2)

Figure 1. Wilt stages of *F. americana* and *F. pennsylvanica*, respectively: a) normal, b) slightly wilted, c) wilted, d) severely wilted, e) nearly dead and f) presumed dead.
Results and Discussion

Figure 2 shows that germination rate decreased with a decrease in substrate water potential. Contrary to expectations, germination of the xeric species, \textit{F. americana}, was more strongly affected by drought stress. These preliminary results suggest that ability to germinate under water stress is not the determinant responsible for the observed distributions of these species. However, to make further conclusions on this, similar germination experiments are to be done with other congeneric species pairs of woody plants. Germination experiments on \textit{Nyssa} spp. and \textit{Vaccinium} spp. are ongoing.

After 7 days of germinating under different water stress treatments, root elongation in \textit{F. pennsylvanica} was more strongly affected than that of \textit{F. americana} (Figure 3). Root growth of \textit{F. pennsylvanica} was greatly reduced under the lowest water potential, while that of \textit{F. americana} was largely unaffected. The more rapid root elongation in the xeric species compared to that in the mesic species may enhance drought tolerance, thus, are more able to establish in water stressed conditions.

After germination and initial establishment, drought continues to act as a filter to plant success. The dry down experiment evaluated the drought tolerance of the xeric and the mesic Fraxinus species. The different wilt stages in \textit{F. americana} and \textit{F. pennsylvanica} closely corresponded to different leaf water potentials (Fig. 5). The leaf water potential of the seedlings decreased linearly with wilting stage. Wilted leaves were brittle and necrosis was observed in the leaf margins and midrib whereas normal leaves appeared to be green and healthy (Fig. 1). Figures 4 and 7 also show that \textit{F. americana} seedlings survived more extreme water stress than \textit{F. pennsylvanica}. The results suggest that \textit{F. americana} are more adapted to dry conditions, thus, allowing a higher survival rate under water stressed conditions. The old and dying leaves were shed while the green wilted leaves without or with limited necrosis remained intact. Surviving plants of \textit{F. americana} developed new leaves that were usually produced at the apex of the shoot while \textit{F. pennsylvanica} seedling resprouted from the middle or the base of the stem (Figure 6). Figure 7 reveals that the shoot of \textit{F. pennsylvanica} is more vulnerable to wilting than \textit{F. americana} at extreme drought stress.
Figure 2. Percent germination of a) *F. americana* and b) *F. pennsylvanica* grown in various water potentials.
Figure 3. Root length increment (mm/day) of *F. americana* and *F. pennsylvanica* 7 days after growing in various water potentials (PEG treatment).

Figure 4. Percent survival of *F. americana* and *F. pennsylvanica* after dry down experiment.
Figure 5. Average leaf water potentials versus wilt stages.

Figure 6. Seedlings of a) *F. americana* and b) *F. pennsylvanica* that survived after subsequent exposure to water stressed conditions.
Figure 7. Percent complete stem survival (a) and percent partial stem survival (b) of *F. americana* and *F. pennsylvanica* after dry down experiment.
Literature Cited


In our ongoing study, Jeffrey Coker (Ph. D. student) used tomatoes for RNA isolations and to probe the wound cDNA library to identify additional wound-up-regulated genes. Much of this work has been published:


Davies E. 2006. “Imagine”. Digitalis 5:2-12


In addition, tomatoes were grown to isolate RNA to provide to Syngenta as part of a Collaborative Research Agreement with Prof Davies. Again, this CRA would not have been possible without the Phytotron. The results are currently being analyzed.

During the year, 8 undergraduate students from NCSU and 2 undergraduate students from St Augustine’s (supported by an NSF grant) worked on this project under my and JeongA’s direction.

Additional Projects:

Wound responses in Arabidopsis. As a follow up to work done by Lisa David (MS graduated) JeongA Yun also used Arabidopsis to study the wound response. Lisa’s work is now being submitted for publication.

Gravity responses in corn. As part of a NASA grant funded to Heike Winter, Imara Perera and Eric Davies, corn plants were grown, subject to gravity stimulation and the pulvini 2 and 5 harvested, cut into upper and lower halves for isolation of RNA. This RNA is currently being used for microarrays and quantitative PCR by Cassie Myburg (post-doc).

We are grateful for the Phytotron personnel and facilities for making this work possible.
THE EFFECT OF MULTIPLE RESOURCE AVAILABILITY ON HYDRAULIC ARCHITECTURE OF REDBAY (*PERSEA BORBONIA*), AND ITS CONSEQUENCES FOR DROUGHT RESPONSE

William Hoffmann and Nikhil Narahari
Department of Botany

The ability of plants to provide its leaves with a constant supply of water is largely dependent on its hydraulic architecture. In particular, the balance of leaf area to root area, and the ratio of leaf area to xylem cross-sectional area will determine the ability to balance transpirational losses with soil water uptake. Light and nutrient availability are known to affect biomass partitioning to roots, stem, and leaves in ways that should influence hydraulic architecture. Although considerable work has focused on how this phenotypic plasticity permits a plant to balance carbon and nutrient uptake, little is known about the consequences for plant water relations.

We hypothesized that changes in biomass allocation that permit a plant to maximize carbon gain under differing resource availability may compromise its ability to tolerate drought. Specifically, we predicted that the phenotypic changes under high nutrient availability and low light availability will reduce the ability of a plant to function under moderate water stress.

Methods

*Persea borbonia* is an evergreen species restricted in North Carolina to barrier islands and maritime forest, and is similar to the more widespread species of redbay, *Persea palustris*.

Seeds of *P. borbonia* were sown into styrofoam cups filled with a mixture of coarse sand and peat moss in a greenhouse maintained at an average daily temperature of 22°C and an average nightly temperature of 18°C. At 16 weeks, seedlings were transplanted into 4” pots, at which time experimental treatments were imposed. Light, nutrients, and water were manipulated in a 2x2x3 factorial design. Light levels were designated as high (direct natural sunlight though clear glass) or low (as above, but reduced with shade cloth permitting x% transmission of photosynthetically-active radiation (PAR)). Nutrients were provided at either high (100%) or low (10% by volume) levels of a complete nutrient solution, which, at full strength, provides 106 ppm N, 10.4 ppm P, 111 ppm K, 54 ppm Ca, 12.4 ppm Mg, 11 ppm Na, 5.0 ppm Fe, 13.19 ppm S, 113 ppb Mn, 240 ppb B, 13 ppb Zn, 5 ppb Cu, 0.03 ppb Co, and 5 ppb Mo.

Water treatments consisted of low, regular, and flooded regimes. Once a week, plants subject to the low water regime received 125 ml of deionized water, in addition to 50 ml of one of the two aforementioned nutrient solutions. For plants assigned to the regular water treatment, nutrient solution was applied once per week, followed by two days during which no water was applied. Subsequently, plants were provided with 125 ml of distilled water every day until the next nutrient treatment. The flooded treatment was identical to the regular water treatment, except that each pot was placed within an individual plastic container that prevented free drainage. The container was sufficiently tall to maintain the water level in the pot the soil surface, while each pot was fitted with a plastic collar to reduce evaporative loss from the soil. For the regular and flooded treatments, the two-day...
interruption in watering following nutrient application was intended to reduce nutrient loss due to leaching. Even so, the water treatments are likely to have influenced nutrient availability, so caution is warranted in interpreting nutrient x water interaction. Similar caution is needed with respect to water x light interactions, since shading is likely to have reduced water loss.

Measurements of transpiration, predawn water potential and midday water potential were performed at several times during seedling development, and again just prior to the destructive measurements of hydraulic conductance. Hydraulic conductances of the whole shoot and whole root system were measured with a high-pressure flowmeter similar to that described by Tyree, using the transient method. Following measurement of hydraulic conductance of the shoot, all leaves were removed and the conductance of the stem was measured. Leaf conductance was calculated from the whole-shoot and stem conductances.

Results

Availability of light, nutrients, and water all significantly influenced whole-plant hydraulic architecture (Figure 1). Leaf-specific conductivity of the whole plant was highest under high light, low nutrients, and flooded soil. Leaf-specific root conductance and leaf-specific stem conductance exhibited similar responses to resource levels (data not shown).

**Figure 1.** Effects of resource levels on whole-plant leaf-specific conductivity.

Much of these effects of resource availability on whole-plant hydraulic architecture can be attributed to changes in allocation patterns. Levels of light, nutrients, and water all significantly affected allocation patterns (Figure 2). The ratio of root area to leaf area was greatest under high light ($F_{1,73}=19.45$, $P<0.001$), low nutrients ($F_{1,73}=10.92$, $P<0.001$), and regular water treatments ($F_{2,73}=4.82$, $P=0.011$).

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In addition to the changes in allocation patterns, changes in tissue-level specific conductivities contributed to the whole-plant responses. Root conductance per root area was significantly greater under flooded conditions than other water treatments ($F_{2,74} = 4.367, P=0.016$), but nutrient and light treatments had no effect ($P>0.25$). Leaf hydraulic conductance was greater under high light ($F_{1,69} = 7.43, P=0.008$) and flooded conditions ($F_{2,69} = 0.013, P=0.013$).
Figure 4. Response of specific leaf conductance (leaf conductance divided by leaf area) to light and water treatments. Nutrient availability had no effect on specific root conductance (not shown).

Discussion.

Resource level had large effects on hydraulic architecture of P. borbonia, with whole-plant leaf-specific conductance being greatest under high light, low nutrients, and flooded soils. These changes reflect changes in whole-plant biomass allocation as well as changes in tissue-level conductances, but the relative importance of these two factors in determining plant response was not the same for all resources. For example, the low leaf-specific conductivity of the whole plant under high nutrient availability (Fig 1) primarily reflects the greater investment in leaves (Fig 2), since there was no effect on root or leaf conductances (Fig 3 & 4).

In contrast, under the flooded treatment, the high whole-plant conductance per leaf area was due entirely to the high leaf and root conductances under this treatment. In fact, had allocation patterns been the only factor influencing hydraulic architecture under the water treatments, plant in the flooding treatment would have had the lowest whole-plant conductances per unit leaf area.

Both allocation and tissue-level responses contributed to the greater whole-plant conductance per unit leaf area under high light. Under high light, plants exhibited greater investment in root area as well as greater leaf conductance. However, allocation patterns had a greater total effect since leaves represented less than 10% of the total plant resistance to water flow.

In the past decade, there has been increasing evidence that hydraulic architecture can exert a strong constraint on the supply of water to leaves. This represents a shift away from the previous focus on the role of environmental factors such as soil water potential and vapor pressure deficit. Even when soil water is abundant, the resistance to water flow through the plant can impose drought-like conditions upon rapidly-transpiring leaves. Decreases in leaf-specific hydraulic conductances, such as those observed here under high nutrient availability, should accentuate this effect.

We are currently in the process of collecting some final data on the harvested individuals, such as wood density, plant mass, and osmotic potential of frozen leaf samples. Other data collected during the study, such as transpiration, leaf water potential, and chlorophyll fluorometry are currently being synthesized to provide a more complete picture of the implications for plant performance.
A UNIVERSAL ROLE FOR INOSITOL 1,4,5-TRISPHOSPHATE-MEDIATED SIGNALING IN PLANT GRAVITROPISM


Department of Botany, North Carolina State University, Raleigh, North Carolina 27695–7612 (I.Y.P., C.-Y.H., W.F.B.); and Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109–7325 (S.B., G.K.M.)

Inositol 1,4,5-trisphosphate (InsP\textsubscript{3}) has been implicated in the early signaling events of plants linking gravity sensing to the initiation of the gravitropic response. However, at present, the contribution of the phosphoinositide signaling pathway in plant gravitropism is not well understood. To delineate the role of InsP\textsubscript{3} in plant gravitropism, we generated Arabidopsis (Arabidopsis thaliana) plants constitutively expressing the human type I inositol polyphosphate 5-phosphatase (InsP 5-ptase), an enzyme that specifically hydrolyzes InsP\textsubscript{3}. The transgenic plants show no significant differences in growth and life cycle compared to wild-type plants, although basal InsP\textsubscript{3} levels are reduced by greater than 90% compared to wild-type plants. With gravistimulation, InsP\textsubscript{3} levels in inflorescence stems of transgenic plants show no detectable change, whereas in wild-type plant inflorescences, InsP\textsubscript{3} levels increase approximately 3-fold within the first 5 to 15 min of gravistimulation, preceding visible bending. Furthermore, gravitropic bending of the roots, hypocotyls, and inflorescence stems of the InsP 5-ptase transgenic plants is reduced by approximately 30% compared with the wild type. Additionally, the cold memory response of the transgenic plants is attenuated, indicating that InsP\textsubscript{3} contributes to gravisignaling in the cold. The transgenic roots were shown to have altered calcium sensitivity in controlling gravitropic response, a reduction in basipetal indole-3-acetic acid transport, and a delay in the asymmetric auxin-induced β-glucuronidase expression with gravistimulation as compared to the controls. The compromised gravitropic response in all the major axes of growth in the transgenic Arabidopsis plants reveals a universal role for InsP\textsubscript{3} in the gravity signal transduction cascade of plants.

In the past year the NCSU Phytotron provided essential space for us to produce several different transgenic plants and study their responses to environmental stimuli. We have not been able to duplicate the required growth conditions with commercially available chambers and could not have done the work without the use of the Phytotron. An abstract from the first of what we anticipate will be many publications describing these plants is pasted below.

*Plant Physiology, February 2006, Vol. 140, pp. 746-760*
SHOOT APICAL MERISTEM AS ALTERNATE TARGET TISSUES FOR MAIZE TRANSFORMATION

Thanh-Tuyen Nguyen, Yufuko Nishimura & Bill Thompson
Department of Botany

As reported earlier, we bombarded immature embryos at an early stage of
development, before the apical dome is covered over by the developing coleoptile. Our
protocol has been optimized to deliver the plasmid DNA to cells in the L1 and L2 layer
while minimizing overall tissue damage. The bombarded embryos develop fully in vitro
and germinated without drug selection to give rise to phenotypically normal plants.

Initially, we discovered that a high percentage of plants derived from bombarded
embryos expressed GUS in their unopened tassels, which were collected for histochemical
assay. This material could not be analyzed further, given the destructive nature of the GUS
assay and the chimeric nature of the plants. Therefore, for the remaining T₀ plants in that
experiment we back crossed them with wild type M37W pollen donors. Seed progeny
from the backcrosses were planted for genetic and molecular analysis. To assess the
frequency of DNA transfer, T₁ plants from each backcross were planted in 6x6 matrices.
Leaf tissues were collected 7-10 days after planting. Simultaneously, we used PCR to
screen for transgene sequences using a pooling strategy followed by tests with individual
progeny to confirm the presence of the transgene. Among seed progeny of four
backcrosses, we recorded frequencies in range of 40%-67% of PCR positive progeny,
scoring for a 353 bp fragment at the junction of 3’ end of the ubiquitin P/I and 5’ end of
uidA gene. Histochemical assay for GUS expression were performed with seedling leaf
segments as well as with segments of mature normal tassels or ear shoots. Although GUS
expression was almost never observed in vegetative tissue, we noted GUS staining in
patchy pattern in segments of mature tassels.

In subsequent experiments, GUS histochemical assays were done with segments of
mature tassels of T₀ plants derived from bombarded embryo. We observed patchy GUS
staining in pollen, anthers and spikelets of assayed tassel segments although, as before,
GUS expression was almost never observed in vegetative tissue. Similarly patchy
expression was observed in tassel segments of T₁ backcross. In the T₁ progeny, PCR
analysis of DNA from leaf tissue usually correlates with staining in the tassel, but there are
some exceptions. Sequencing of PCR products from genomic DNA of one transformation
event shows a perfect complement to the transgene sequence of 353 bp at the junction of
the 3’ end of Ubi P/I and 5’ end of GUS, and of 520 bp at the 3’ end of GUS. However,
only one out of three putatively transformed lines analyzed by Southerns contained
fragments hybridizing to probes for the middle and 3’ end of the uidA gene.

One hypothesis to explain the non-uniform staining data is that the transgene may
be effectively silenced by PTGS during vegetative growth and later (partially) reactivated
in the reproductive tissue. A similar phenomenon was reported in tobacco (Mitsuhara et
al, 2002.) In this case, however, one would expect the (silenced) transgene to be present
uniformly in all cells of those backcross progeny. Until we have completed additional
molecular analyses, we cannot be confident that this is the case in all our lines. One
alternative hypothesis is that some type of persistent endophyte may have been transformed during microprojectile bombardment, and that this endophyte has a variable distribution in different individual plants. More data will be required to determine whether the transgenes we have characterized are integrated into known maize sequences.

Molecular analysis of T1 backcross lines from more putative transformation events will be performed to provide more definitive evidence of germline transformation by our reported method.

REFERENCE:

FUNCTION AND REGULATION OF ARABIDOPSIS PEPTIDASES

Utku Avci and Candace Haigler

My laboratory used the Arabidopsis growing room in the Phytotron to support the research of Ph.D. graduate student, Utku Avci (enrolled in Crop Science). He was working on a grant from the NSF Integrative Plant Biology Program in a project that was collaborative with Dr. Eric Beers at VPI. Arabidopsis plants with altered expression of peptidases and with reporter genes under the control of peptidase promoters were grown in the Phytotron under standard conditions for Arabidopsis. The availability of this dedicated growing room is of substantial help for Arabidopsis research because variable growth conditions (such as usually exist on lighted racks in laboratory rooms) can cause changes in both the developmental timing and phenotypes of growing plants. Under standardized and constant conditions, the repeatability of experiments and results is greatly increased. In 2005, Utku was successful in performing electron microscopic immunolocalization of the peptidase, as reflected in the published abstract listed below. His data are consistent with these particular cysteine proteases having a role in tracheary element autolysis, but, contrary to dogma, they appear to remain in the cytoplasm until their degradative role commences instead of being loaded into the vacuole. There is considerable interest in the cellular trafficking of cysteine proteases and in all aspects of tracheary element differentiation, so these results together with analysis of a double knock-out in two proteases, will be published in a high quality journal in 2006.

One abstract was published:

EFFECT OF DAY/NIGHT GROWTH TEMPERATURE ON THE STABILITY OF OLEIC ACID CONCENTRATION IN SOYBEAN SEEDS

J. Burton and M. Villagarcia

Soybean oil has poor oxidative stability. Stability can be improved by increasing the percentage of oleic acid in the oil. Soybean breeders are busily trying to breed a genotype with 3 % linolenic and a mid-level (50-60%) oleic acid. Genotype N98-4445A is the result of this breeding effort with both chemical traits, low linolenic and mid-oleic, in its oil composition (Burton, et al. 1983; Carver, et al., 1986; Wilson, et al., 2001). However, environment plays also an important role in defining the ratio of fatty acids in soybean oil. For example, the ratio of polyunsaturated to monounsaturated fatty acids in soybean oil is known to decrease with high temperature (Rebetzke et al., 1996). An important goal is to identify sources of mid or high oleic acid that are more stable under differing temperatures regimes.

In 2004, 77 soybean plants introductions (PIs), previously identified as having above average oleic acid concentration, were evaluated in the phytotron under two different night temperature regimens (day/night = 30/28 and 30/20 °C). Genotypic responses to dark period temperature were extremely diverse, with changes in oleic acid ranging from -8.55 to +19.25 %, and linolenic acid from -4.1 to +1.2. Based on these results, our objective in this study was to determine the effect of dark temperature on fatty acid seed composition of selected soybean genotypes during seed fill and maturation (R5-R8).

In 2005, 14 of the 77 PIs plus 5 checks (Dare, Dunfield, N98-4445A, PI 123424, N87-2117-3) were tested under the same temperature regimes as the first study. These genotypes selected for this experiment were based on their change in response in oleic acid concentration when plants were exposed to the temperature treatments regimes during seed fill and maturation. This study was conducted following a similar procedure as the first experiment (Burton, 2004) with the following exceptions: two H
chambers were used, plants were grown in 20-cm plastic pots, and three seeds were planted and thinned to one per pot after emergence.

All genotypes were grown under the same day/night temperature (30/28 °C) until all plants reach the beginning of seed filling (about 50 days after planting- R5 stage). At this point, night temperature in the second chamber was lowered to 20 °C, while the first chamber was kept at 28 °C. Plants in both chambers were grown to maturation. Pods were hand-harvest as they mature. Fatty acid composition of the seed oil was determined on five seeds samples from each plant by gas chromatography.

The experimental design was in a randomized complete block design with 3 reps. Data were statistically analyzed using the GLM procedure from SAS.

Results and Discussion

Dark temperature treatments during seed fill and maturation (R5-R8) altered the fatty acid composition of the seeds considerably (Table1). A decrease in night temperature from 28 °C to 20 °C resulted in no significant change in palmitic acid, lower oleic acid and higher linolenic acid concentration when averaged across all genotypes. When the oil compositions of the individual genotypes were compared between the two chambers, the only fatty acid that did not change significantly in the extracted seed oil was palmitic acid. However, stearic, linoleic, and linolenic acid concentration increased. On the other hand, the concentration of oleic acid decreased considerably in soybean plants grown in a cooler night temperature. The same trend of response to temperature effect was observed in the fatty acid composition in the oil of the seeds in the control genotypes. Genotypes N98-4445A and N87-2117-3 showed greater changes in their fatty acid composition compared to the other genotypes. Cooler night temperature resulted in a decrease in oleic acid concentration of 300 mg g-1 (when averaged over both genotypes).
Although similar changes in fatty acid composition were observed in the first study, differences among genotypes were slight and inconsistent for oleic acid concentration when we compared both studies (Table 2). However, these data provides information that warmer temperatures at night during seed fill and maturation are associated with higher concentration of oleic acid, and a decrease in the saturated and polyunsaturated fatty acids of soybean seed oil.

References


Table 1. Seed fatty acid composition of soybean exposed to two dark period temperature regimes (28 and 20 °C) during seed fill and maturation. Day temperature was kept constant in both chambers (30 °C)

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N87-2117-3 8.8 10.6 -1.8 2.5 6.2 -3.7 25.0 50.1 -25.0 3.0 4.2 1.2
Dunfield 10.1 11.2 -1.1 2.5 5.0 -2.5 36.9 49.6 -12.7 5.0 10.5 5.5
PI-123440 9.1 9.6 -0.5 2.7 5.7 -3.0 38.4 52.2 -13.8 4.2 5.4 1.2

**Mean** 10.3 10.8 -0.5 2.5 5.0 -2.5 36.4 48.3 -11.9 5.8 8.2 2.4
Table 2. Seed oleic acid exposed to two dark period temperature regimes (28 and 20 °C) during seed fill and maturation for 2004 and 2005. Day temperature was kept constant in both chambers (30 °C) during both years.

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TEMPERATURE AND GLUFOSINATE RATE EFFECTS ON PALMER AMARANTH (*Amaranthus palmeri*) AND PITTED MORNINGGLORY (*Ipomoea lacunosa*) EFFICACY

Wesley J. Everman, Walter E. Thomas, Whitnee L. Barker, and John W. Wilcut

INTRODUCTION

Palmer amaranth (*Amaranthus palmeri*) and pitted morningglory (*Ipomoea lacunosa*) are troublesome and competitive weeds in southern row crops (Webster 2004). Glufosinate formulations provide excellent control of pitted morningglory (6 inch) and other annual morningglory species while control of Palmer amaranth and other pigweed species is limited to small (<3 inch) plants (Anonymous 2006a, 2006b). Glufosinate was registered for use in glufosinate-resistant corn and cotton in 1997 and 2004, respectively. Glufosinate, a non-selective herbicide, inhibits glutamine synthesis leading to ammonia accumulation and eventual death. Glufosinate is a contact herbicide requiring thorough or near complete coverage to ensure good control. Glufosinate has no residual activity with no rotational or replant concerns (Anonymous 2006).

Cotton has limited postemergence herbicide options; therefore, with Palmer amaranth developing resistance to the alternative POST options of glyphosate and acetolactate synthase herbicides (Heap 2006), the introduction of glufosinate-resistant cotton provides a new weed management tool for cotton farmers. A new herbicide program is needed to manage resistant Palmer amaranth and the use of glufosinate with glufosinate-resistant crops may provide cotton farmers with the tools needed to avoid or delay resistance. However, control of amaranth species has been reported to be erratic (J. W. Wilcut, personal communication) with glufosinate. Previous studies with glufosinate indicate that its efficacy depends on various environmental conditions, weed species treated, and application rates (Carlson and Burnside 1984). Temperature and humidity in particular seem to have an effect on glufosinate efficacy. Anderson et al. (1993) demonstrated that both temperature and relative humidity (RH) have a considerable effect on the activity of glufosinate in barley (*Hordeum vulgare* L.) and in green foxtail [*Setaria viridis* (L.) Beauv.]. Coetzer et al. (2001) reported increasing control of amaranth species with glufosinate as humidity increased, with differential response to temperature among...
amaranth species. Similarly, Kumaratilake and Preston (2005) reported reduced control of wild radish with glufosinate at 5/10 C when compared to 15/20 or 20/25 C. Absorption was not affected by humidity or temperature, however translocation was greater in plants grown at higher humidity (Coetzer et al. 2001) and a low light intensity at 20/25 C (Kumaratilake and Preston 2005). Basipetal translocation of glufosinate was greatly increased by higher temperature (Kumaratilake and Preston 2005). Therefore, the effects of environmental factors on glufosinate efficacy need to be further investigated in susceptible (morningglory) and moderately tolerant (amaranth) species. Experiments were conducted to determine the effects of temperature and glufosinate rate on Palmer amaranth and pitted morningglory.

**MATERIALS AND METHODS**

The experiment was conducted in the North Carolina State University Southeastern Plant Environmental Laboratory, Raleigh, North Carolina. Five chambers, 3 or 9 m$^2$ with a height of 2.1 m, were used to grow Palmer amaranth and pitted morningglory under optimum and suboptimum temperature regimes of 14/10 ± 2 C, 18/14 ± 2 C, 22/18 ± 2, 26/22 ± 2 C, and C34/26 ±2 C. Lighting was provided by a combination of fluorescent and incandescent lamps (380 umol/m$^2$/s photosynthetic photon flux), and all chambers were set for a 14-hr photoperiod. Both species were planted in pots with a diameter of 25 cm. Pitted morningglory seeds were pre-germinated and transplanted one per pot, while Palmer amaranth seeds were sown in the pots and thinned to one per pot. Each pot contained a substrate of steam-sterilized and washed #16 gravel and a proprietary blend of peat and vermiculite. All pots were watered with a standard nutrient solution twice daily throughout the study. All plants were grown at optimal temperature until one day before initiation of the study, at which time they were moved to their respective temperature for the duration of the study. Plants were treated with glufosinate at 0.21, 0.42, and 0.84 lb ai/A (0.5X, 1X, and 2X labeled rates). Non-treated controls were included for comparison for each species and temperature. Treatments consisted of a factorial arrangement of two species (Palmer amaranth and pitted morningglory), five day/night temperature regimes (14/10, 18/14, 22/18, 26/22, and 32/26), and four herbicide rates (0, 0.5, 1, and 2X). Photosynthetic net assimilation
rate (NAR), leaf number, plant height, and visual control ratings were measured 1, 3, and 7 d after treatment (DAT). Photosynthetic NAR was measured on the highest fully expanded leaf using a LI-COR LI-6400 portable photosynthesis system 1 DAT. Total above ground fresh biomass and dry biomass were measured at 7 DAT. Above-ground dry biomass was determined after drying at 65C for 5 d.

The experimental design was a randomized complete block design arranged in a factorial treatment arrangement. The experiment was conducted twice. Data were subjected to an analysis of variance (ANOVA) using PROC GLM with partitioning appropriate for a two by four by five factorial treatment arrangement of species, temperature regime, and herbicide rate.

RESULTS AND DISCUSSION

There was a significant temperature by herbicide rate interaction for Palmer amaranth and pitted morningglory control at 1 and 3 DAT, fresh weights, and dry weights. As temperature increased, control of pitted morningglory and Palmer amaranth with glufosinate at 0.42 lb ai/A increased for all herbicide rates (Tables 1 and 2). Pitted morningglory control was at least 75% after any glufosinate application when plants were kept in 22/18 to 32/26 C day/night temperatures (Table 1). Control of pitted morningglory was statistically similar using the 1X and 2X rate for all temperature regimes, while control with the 0.5X rate was significantly lower for all temperatures except the 26/22 C regime (Table 1). Non-treated pitted morningglory fresh and dry weights increased as temperature increased (Table 1). For Palmer amaranth, as the rate increased within a temperature regime, the control also increased at both 1 and 3 DAT (Table 2). Fresh and dry weight analysis showed an increasing trend in fresh and dry weight for untreated plants as temperature increased (Table 2). A herbicide main effect was detected for pitted morningglory and Palmer amaranth control at 7 DAT (Table 3). Pitted morningglory control was not significantly different (≥ 93%) for all rates (Table 3). However, Palmer amaranth control was significantly different at all herbicide rates 7 DAT (Table 3). Glufosinate applied at 0.21, 0.42, and 0.84 lb ai/A controlled Palmer amaranth 84, 90, and 94%, respectively, at 7 DAT. These results indicate a rate response in Palmer amaranth regardless of temperature. This may be due to the slowed
metabolism of the plant and delayed translocation or action of glufosinate in the cooler temperatures. Coetzer et al. (2001) observed reduced translocation of glufosinate in Palmer amaranth as temperature was decreased. Similarly, wild radish control was poorly controlled in day/night temperatures of 10/5 C compared to plants grown in 20/15 and 25/20 C (Kumaratilake and Preston 2005). The differences in control of Palmer amaranth and pitted morningglory grown at different temperatures may also be explained by physiological differences in the two species. Ridley and McNally (1985) found differences in susceptibility of seven plant species to glufosinate, possibly due to different ratios of the two isoenzymes of glutamine synthetase that occur in plants.

Table 1. Pitted morningglory control and weight with glufosinate as influenced by temperature and glufosinate rate. Means are averaged over runs.

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<th>Control 3 DAT</th>
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Table 2. Palmer amaranth control and weight with glufosinate as influenced by temperature and glufosinate rate. Means are averaged over runs.

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Table 3. Palmer amaranth and pitted morningglory control at 7 DAT as affected by herbicide rate. Means are averaged over runs and temperatures.

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LITERATURE CITED


MESOFEAUNAL CONTROLS OVER PATHOGENIC FUNGI IN SOIL

Shuijin Hu and Ron Qu
Department of Plant Pathology

The Soil Ecology Lab in Plant Pathology Department examined the effects of diversity and activities of mesofauna on soilborne pathogenic fungi in 2005. Two growth chamber experiments were conducted in growth chambers under controlled environments in Phytotron Facility, using pepper (or tomato)–*Pythium ultimum* (or *Phytophthora capsici*) pathosystems. The experiments were designed to assess how mesofauna contribute to the suppressiveness of the populations of fungal pathogens (*P. ultimum* and *P. capsici*) and their activities (i.e., diseases in pepper or tomato) in soil. Results obtained indicated that soil fauna significantly contributed to the observed suppression of disease incidence in two organic soils. Introduction of fungivorous nematodes (e.g., *Aphelenchus avenae*) and collembolans (e.g., *Hypogastrura perplexa*) can significantly reduce the populations of soilborne fungal pathogens and their activities. However, suppression efficiency is significantly different among the mesofaunal species, and soil structure and faunal feeding behaviors critically impact the suppression efficiency. Moreover, mixtures of soil mesofaunal species enhance pathogen and disease suppression in soil.
1. In 2005, we used the greenhouse and growth chambers in the Phytotron to evaluate transgenic tall fescue plants for resistance to fungal diseases or drought tolerance. Approximately 20 plants harboring one of five different transgenes were inoculated with *Rhizoctonia solani* or *Magnaporthe grisea* to test their resistance to the brown patch or gray leaf spot diseases. Three replicates were performed. A majority of the plants showed significant reduction in lesion size and disease incidence when inoculated with the gray leaf spot pathogen. About one third of the plants challenged showed significant resistance to the brown patch disease. Moreover, six out of the 30 transgenic tall fescue plants tested showed an improved drought tolerance. We are currently performing a series of physiological assays to further evaluate these plants in the Phytotron.

2. In 2005, the Phytotron provided space for us to grow transgenic rice plants to evaluate the expression strength and pattern of a rice gene promoter we isolated. In that project, we also evaluated the effect of an intron on the promoter expression level and pattern. The uniformity of the phytotron environment allowed us to obtain reliable data, and the experiments are nearly finished.

3. We also grew some transgenic Arabidopsis plants to evaluate two genes that may be critical to the intron-mediated enhancement of gene expression in plants.

4. We started growing wheat plants to provide experimental materials for a wheat transformation project aiming to improve its drought tolerance.

In 2005, two of our publications used the data from the plants grown in the phytotron. We have acknowledgement to the Phytotron at the end of the papers. They are:


The well-managed NCSU Phytotron is an extremely useful facility for our experiments. We appreciate very much the help we received in the past year from the Phytotron staff and the facility.
A MOLECULAR GENETIC APPROACH TO UNDERSTANDING THE
CONTROL OF SECONDARY WALL CELLULOSE SYNTHESIS IN COTTON
FIBER
Bir Singh, Raj Balasubramanian, Utku Avci, and Candace Haigler

We are performing functional genomics research based on 3,420 G.h.fbr-sw (Gossypium hirsutum fiber secondary wall) putative unigenes expressed during the secondary wall phase of cotton fiber development. The goal is to learn more about molecular controls of secondary wall cellulose deposition in cotton fiber. Knowledge gained through this project is expected to support improvement of cotton fiber through biotechnology or marker-assisted breeding. The project involved the P.I., two Ph.D. Research Associates, and Ph.D. student, Utku Avci.

The G.h.fbr-sw unigenes were derived from sequencing of a suppression subtractive hybridization (SSH) library biased toward genes that are expressed in fiber at 20 days post anthesis (DPA; secondary wall) compared to 6 DPA (primary wall) (Haigler et al. 2005). In 2005, the Phytotron 26°/22°C greenhouse was used to grow cotton to validate by quantitative PCR the results from a spotted cDNA microarray experiment including G.h.fbr-sw unigenes, which was designed to compare gene expression in 6, 10, 20, and 24 DPA. The tight temperature control in this greenhouse was critical for repeatability in the timing of cotton fiber development, which is quite sensitive to temperature variation. Through examination of fiber grown in the Phytotron greenhouse to validate its developmental state, which was important to results described in the published abstract listed below, we also discovered novel mechanisms that were described in the NCSU invention disclosure listed below. In another use of cotton fiber grown in the Phytotron, samples were sent to Dr. William G.T. Willats, Associate Professor, The University of Copenhagen. He will perform large scale testing for cell wall components that can be recognized by a panel of antibodies to plant cell wall components. This information will aid interpretation of microarray results, as well as provide a new kind of comprehensive information about changes in the cotton fiber wall during development.

In another functional genomics approach, we used the Phytotron Arabidopsis growth chamber in 2005. We are exploring the function of selected members of the G.h.fbr-sw unigene set through analysis of T-DNA mutants of Arabidopsis. Many T-DNA mutants were grown for genotypic screening and preliminary analysis of phenotypes. The constant growth conditions in this chamber were important to insure repeatability of phenotypes. This work is continuing in 2006.

One full paper that describes the basis of this research project was published:

One abstract was published including emerging results:
Haigler CH, Secondary wall stage cotton fiber provides an optimum platform for analysis of gene expression related to cellulose synthesis. Abstract Book: Biosynthesis of Plant Cell
One Invention Disclosure was filed:
NCSU, #06-022, Novel Mechanisms Regulating Cotton Fiber Development (2005)
UNCOUPLING PRIMER AND RELEASER RESPONSES TO PHEROMONE IN HONEY BEES

Christina M. Grozinger, Patrick Fischer* and Jacob Hampton*

Departments of Entomology and Genetics
W.M. Keck Center for Behavioral Biology
North Carolina State University
* These authors contributed equally

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Pheromones produce dramatic and stereotyped changes in behavior and physiology in a wide variety of species (reviewed in Wyatt 2003). They can act as releasers, eliciting responses within a matter of seconds or minutes, or primers, causing long-term changes in behavior or physiology over a period of days. However, while the responses to pheromones are classically considered to be innate and hard-wired (Wyatt 2003), there are multiple examples in which physiological changes modulate the behavioral response to a pheromone (Robinson 1987; Pham-Delengue et al. 1993; Gadenne et al. 2001; Pankiw 2004). In this study, we compared the releaser and primer effects of queen mandibular pheromone on worker honey bees, to determine if it was possible to uncouple these responses under different conditions. If so, this would indicate that the brain uses different neural or gene networks to produce these two types of responses.

The most important and best-characterized pheromone in honey bees is produced by the queen; this multigland, multicomponent pheromone elicits a variety of responses in worker bees (Winston and Slessor 1998; Keeling et al. 2003). Five of these components (termed queen mandibular pheromone or QMP) produce many of the effects of a live queen. QMP acts as both a releaser and primer pheromone. As a releaser pheromone, QMP induces a retinue response, in which young bees are attracted to the queen (or a lure impregnated with QMP) and lick and antennate her. It also stimulates forager bees to initiate foraging activity. As a primer pheromone, QMP inhibits the worker ovary development in young bees (Hoover et al. 2003), so that they remain a sterile caste, and delays the nurse-forager transition. QMP also reduces the responsiveness of young bees to a sucrose reward, as measured by the proboscis extension response (PER). Recently, it has also been demonstrated that long-term QMP exposure stably alters expression of 19 genes in the brains of young workers bees, including the honey bee ortholog of kruppel-homolog 1 (kr-h1). Kr-h1 is a zinc-finger transcription factor, which is critical for a variety of developmental processes in Drosophila melanogaster (Bhat 1998; Pecasse et al. 2000), and has recently been associated with Drosophila courtship (Lawniczak and Begun 2004; Mackay et al. 2005). However, its molecular functions in adult brains are unknown.

We conducted two studies to determine if the primer and releaser responses to QMP could be uncoupled. First, we tested the effects of two of the individual components of QMP, 9-ODA and 9-HDA, on both the retinue response and downregulation of kr-h1 in the honey bee brain. Secondly, we tested the effect of treatment with a juvenile hormone
analog (methoprene) on the retinue response and the ability of QMP to reduce PER response to sucrose rewards. We found that the full QMP blend and 9-ODA elicited a retinue response, while 9-HDA did not. However, expression of kr-h1 was significantly downregulated in bees exposed to QMP, 9-ODA and 9HDA. We found that methoprene treatment had no effect on the retinue response. However, methoprene did block the effect of QMP exposure on reducing sucrose responsiveness. Alone, methoprene has no effect on QMP response.

Thus, the primer (gene expression, sucrose response) and releaser (retinue response) effects of QMP can be uncoupled. This suggests that these behaviors are regulated by different molecular or neural pathways in the brain.

Acknowledgements. We would like to thank the NCSU Phytotron for providing the incubator facilities necessary for maintaining cages of bees under constant humidity, temperature and red light. We would also like to thank Jennifer Keller and Joshua Summers for expert assistant with maintaining honey bee colonies at the Lake Wheeler Bee Facility. This work was supported by a grant from the NIDCD grant to Gene Robinson (subaward to CMG, 1 R01 DC006395), and an NCSU Undergraduate Research Fellowship to PF.

References.

Bhat KM (1998) frizzled and frizzled 2 play a partially redundant role in wingless signaling and have similar requirements to wingless in neurogenesis. Cell 95(7): 1027-1036.


FUNCTIONAL ANALYSIS OF RECEPTOR KINASES REGULATING ARABIDOPSIS GROWTH AND DEVELOPMENT

Steven D. Clouse, Department of Horticultural Science
Michael B. Goshe, Department of Biochemistry

We used the Arabidopsis growth room of the phytotron to maintain and propagate a variety of transgenic lines and mutants essential for the analysis of the molecular mechanisms of leucine-rich repeat receptor-like kinases involved in regulating plant growth and development. The work involved two postdoctoral scientists, Dr. Srijeet Mitra and Dr. Xiaofeng Wang, and a laboratory specialist, Mr. Gregory Scott. Funding for the project was obtained from the National Science Foundation Arabidopsis 2010 program and the USDA/NRI Competitive Grants Program.

The Arabidopsis genome encodes more than 200 Leucine-Rich Repeat Receptor-like kinases (LRR RLKs) with an organization of functional domains similar to that of animal receptor kinases. Several LRR RLKs are known to be critical elements in signaling pathways regulating plant development and response to the environment, but the biological functions of most members of this large family of putative receptors remain unknown. This project is acquiring fundamental biochemical knowledge of the kinase domains of all Arabidopsis LRR RLKs by using Gateway cloning and high-throughput liquid robotics for \textit{in vitro} analysis of autophosphorylation activity, substrate preference and pair-wise interactions. A proteomic analysis of membrane proteins isolated from Arabidopsis plants grown under a variety of physiological conditions is being used to identify a subset of 30 LRR RLKs for detailed analysis of \textit{in vivo} autophosphorylation sites by mass spectrometry. The functional significance of selected phosphorylation sites has been examined using genetic and biochemical approaches, with plants grown in the phytotron. Information about the project, including a list of genes studied can be obtained at http://www.cals.ncsu.edu/hort_sci/faculty/clouse.html.

Work with plants grown in the Phytotron led to two publications in 2005:


SEED GERMINATION OF SEABEACH AMARANTH  
(AMARANTHUS PUMILUS) IN RESPONSE TO TEMPERATURE,  
LIGHT, AND GIBBERELLIN A₃ TREATMENTS  

Daniel S. Norden, Frank A. Blazich,  
Stuart L. Warren, and David L. Nash  

Seeds of seabeach amaranth (Amaranthus pumilus Raf.), a species federally listed as "threatened," that had been in storage at 4C (39F) for approximately 1 year (harvested September 2003) and freshly harvested seeds (October 2004) of the species were soaked in November 2004 in solutions of the potassium (K) salt (K-salt) of gibberellin A₃ (K-GA₃) at 0, 100, 500, or 1000 mg/liter (ppm) for 24 hr in darkness. After treatment, seeds were germinated at 25C (77F) or an 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness) or 16 hr. Germination was recorded every 3 days for 30 days. Both groups of seeds responded similarly despite the storage and age differences between the groups. The 2003 seeds had greater vigor and viability, and these data are discussed. Regardless of germination temperature and photoperiod, nontreated seeds [0 mg/liter (ppm) K-GA₃] did not germinate. When germinated at 25C (77F) the response of seeds to K-GA₃ treatment was linear for both photoperiods with significantly greater total (30-day) germination occurring in the dark for seeds treated with 100, 500, or 1000 mg/liter (ppm) K-GA₃. At 25C (77F), the greatest total germination (84%) was observed for seeds treated with 1000 mg/liter (ppm) K-GA₃ and maintained in darkness, whereas for seeds exposed to a 16-hr photoperiod, maximum germination was 72%. At 30/20C (86/68F) the response to K-GA₃ was quadratic with maximum germination at predicted rates of 882 and 875 mg/liter (ppm) K-GA₃ (88% and 92%, respectively) for photoperiods of 0 and 16 hr, respectively. Treatment of non-stratified seeds of sea beach amaranth with K-GA₃ removed physiological (embryo) dormancy eliminating the need for stratification (moist-prechilling). Treatment also reduced sensitivity of the seeds to light, and appeared to broaden the range of temperatures for germination. A journal manuscript dealing with this research has been submitted for publication.
2005 Publications Related to Phytotron Usage

Proceedings


Refereed Journal Articles


IMPACT OF FLOWER REMOVAL AND LIGHT AND TEMPERATURE STRESSES ON ACIDIFICATION OF SUBSTRATE BY GERANIUM (PELARGONIUM X HORTORUM BAILEY)

Matthew D. Taylor & Paul V. Nelson
Department of Horticulture Science, North Carolina State University, Raleigh, NC 27695-7609

Jonathan M. Frantz
USDA-ARS, Toledo, OH 43606

The cause of sudden substrate pH decline by geranium (Pelargonium x hortorum Bailey) is unknown. Geraniums growing at a substrate pH of 6 or higher suddenly cause substrate pH to shift downward one to two units within a week or two, with values as low as 3.8 reported. In organic based soilless media the pH has a large effect over nutrient availability and crops are often devastated from low pH problems of iron and manganese toxicities and calcium and magnesium deficiencies. Published reports indicate that this response can be influenced in other plants by temperature and light extremes. The first of five experiments compared plants with all flowers removed to plants that were allowed to flower. Experiment 2 compared plants grown at 4 light levels (105, 210, 450 and 1020 µmol·m⁻²·s⁻¹). Experiment 3 compared plants grown at 4 temperatures (14/10, 18/14, 22/18 and 26/22º C day/night). Experiment 4 was a repeat of experiment 1 and experiment 5 was a factorial combining the 3 highest light levels and the 3 highest temperature levels. Plants allowed to form flowers had a final substrate pH of 6.3 compared to 5.7 for plants where flowers were removed. With increasing increments of temperature, substrate pH declined from 6.8 to 4.6 and with increasing light intensity from 6.1 to 4.8. There was no effect of flower removal in experiment 4. Light and temperature had no consistent effects in Experiment 5 throughout 46 days after planting with most pH values remaining in the acceptable range of 5.6 to 6.1. By 60 days, temperature treatments began to segregate with pH being highest in the low temperature treatments and lowest, down to 5.5, in the highest temperature treatments. High temperature stimulated geranium acidification in both experiments, with the effect more severe in the first experiment. The flowering and high light effects were not duplicated in the second trial. This indicates that an additional factor is involved in expression of the light, temperature, and flowering control of acidification.
SEED GERMINATION OF PINKSHELL AZALEA (RHODODENDRON VASEYI) AS INFLUENCED BY LIGHT AND TEMPERATURE

Lela C. Walker, Anthony V. LeBude, Frank A. Blazich, and Joseph E. Conner

Introduction

The deciduous pinkshell azalea (Rhododendron vaseyi Gray) is a rare, ericaceous species endemic to five counties in western North Carolina (5). Plants produce attractive, pink to sometimes white, woodsy smelling flowers that appear with leaf development in May and June. The primary habitats of this plant are swamps, bogs, ravines, and stream banks in oak and coniferous forests at elevations of 914 to 1676 m (3000 to 5500 ft) (4). Unfortunately, construction of vacation homes and housing developments in addition to stream desecration have decreased these habitats with subsequent loss of plants (5). As a consequence, protocols need to be developed for propagation of the species to ensure survival and one obvious method of propagation would be sexual (seed) propagation. Thus, the following research was conducted to study the influence of light and temperature on seed germination of pinkshell azalea.

Materials and Methods

On September 2, 2005, mature seed capsules (fruits) were collected from two native populations of open-pollinated plants of pinkshell azalea. Capsules of the first population were collected on Mount Pilot in Transylvania County, North Carolina, and capsules of the second were collected along the ridgeline between Jackson and Transylvania counties at the intersection of Highway 215 and Charley’s Creek Road. All capsules were pooled.

Capsules were dried at 21 °C (70 °F) until September 23, 2005, when seeds were released using a mortar and pestle. Chaff and other debris were removed and cleaned seeds were stored at 4 °C (39 °F) in a sealed glass vial until the experiment was initiated on October 9, 2005.

Graded seeds were removed from storage and placed in covered 9-cm (3.5 in) diameter glass petri dishes, each dish containing two pre-soaked germination blotters moistened with tap water. After placement of seeds in the petri dishes, half the dishes were designated for germination at 25 °C (77 °F) and the other half for germination at an 8/16 hr thermoperiod of 30/20 °C (86/68 °F). All dishes were placed in black sateen cloth bags and the seeds were allowed to imbibe overnight at 21 °C (70 °F). Beginning at 8 AM the following day, the bags were randomized within two C-chambers, one set at 25 °C (77 °F) and the other at an 8/16 hr thermoperiod of 30/20 °C (86/68 °F). Temperatures within chambers varied within ± 0.5 °C (0.9 °F) of the set point.

Within each temperature regime, seeds were subjected daily to the following eight photoperiods: 0 (total darkness) 1/2, 1, 2, 4, 8, 12, or 24 hr. All photoperiod treatments, with the exception of the 0 and 24 hr treatments began with the transition to the high temperature portion of the cycle. Growth chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400-700 nm) of approximately 40 µmol·m²·s⁻¹ (3.2 klx).
Daily photoperiod treatments were regulated by removal and placement of the petri dishes into black sateen cloth bags. Petri dishes for the 24 hr photoperiod treatment remained continuously unbagged in the chambers. Petri dishes for the 0 hr (total darkness) treatment remained bagged throughout the experiment and germination data were recorded under a green safelight. Germination blotters were kept moist with tap water throughout the experiment. Within a temperature regime, photoperiods were replicated four times with a replication consisting of a petri dish containing 50 seeds. Germination counts were recorded every 3 days for 30 days, and seeds showing signs of decay were removed from the dishes. A seed was considered germinated when radicle emergence was $\geq 1$ mm (0.04 in) in length. Percent germination was calculated as a mean of four replications per treatment. Within each temperature, data were subjected to analysis of variance procedures and regression analysis (SAS v. 9.1, SAS Institute, Cary, NC).

**Results and Discussion**

Light was required for seed germination of pinkshell azalea regardless of the temperature (Figs. 1 and 2). This is also true for other species of rhododendron (*Rhododendron* L. spp.) (1, 2, 3). For both temperatures, germination was a function of photoperiod. A significant linear and quadratic effect of photoperiod on germination was shown by an increase in germination with an increase in photoperiod (Fig. 1). The 8/16 hr thermoperiod of 30/20 °C (86/68 °F) partly compensated for the light requirement. This is illustrated by comparing germination percentages between 25 °C (77 °F) and 30/20 °C (86/68 °F) for photoperiods of 4 (2% vs. 4%), 8 (3% vs. 9%), 12 (5% vs. 25%), and 24 (28% vs. 54%) hr (Figs. 1 and 2). A similar effect has been reported for other species of rhododendron (1, 2, 3).

Overall germination was low for this species. Low germination for this experiment appears to have resulted from poor seed viability and vigor, which may explain its limited range in North Carolina. However, it is possible that this may have been just an anomaly caused by unfavorable environmental conditions in 2005 during seed development. A subsequent germination study was conducted using seeds also collected in Fall 2005 from a more northerly location in North Carolina. Despite lower overall germination in this second experiment, the effects of light and temperature were similar to data reported herein. Additional seed germination studies of pinkshell azalea will be conducted in Fall 2006 using freshly harvested seeds.

**Literature Cited**


![Graph showing cumulative seed germination](image1)

**Fig. 1.** Cumulative (30-day) seed germination of pinkshell azalea as influenced by photoperiod and temperature. The linear and quadratic effect of photoperiod on germination percentage was significant for both temperatures.

(A) Germination at 25 ºC  
(B) Germination at 30/20 ºC

![Graph showing influence of germination temperatures](image2)

**Fig. 2.** Influence of germination temperatures of (A) 25 ºC (77 ºF) and (B) 30/20 ºC (86/68 ºF) and photoperiods of 0 (total darkness – solid square), 1/2 (open square), 1 (solid triangle), 2 (open triangle), 4 (solid circle), 8 (open circle), 12 (solid diamond), and 24 hr (open diamond) on seed germination of pinkshell azalea over 30 days.
EFFECTS OF PYRACLOSTROBIN ON CREEPING BENTGRASS PHYSIOLOGY AND *PYTHIUM VOLUTUM* MYCELIAL GROWTH.


*Department of Plant Pathology
Department of Crop Science

Introduction:

Since 2002 an uncharacterized root pathogen of creeping bentgrass has plagued the Southeastern United States. After examining many samples it was evident that a *Pythium* species was the causal agent of the root disease. Sixty-five isolates were collected from North Carolina and Virginia and after identification of the isolates, *Pythium volutum* appeared to be the causal agent of the root disease. Symptoms observed in North Carolina resembled symptoms described by Hodges and Coleman in Iowa (1). In Iowa the disease was called Pythium root dysfunction. The stand symptoms in North Carolina are very similar to the symptoms documented in Iowa by Hodges and Coleman. Therefore, the uncharacterized root disease of creeping bent grass in North Carolina is likely Pythium root dysfunction.

Very little is known about the disease cycle and epidemiology of Pythium root dysfunction and there is no information about chemical control of Pythium root dysfunction. To rectify this, fungicide trials were established across the state of North Carolina to determine fungicide efficacy against Pythium root dysfunction. Pyraclostrobin (BASF’s Insignia) provided the best control of Pythium root dysfunction in all trials. Pyraclostrobin is a QoI fungicide, which is not known for *Pythium* control. The more typical fungicides used for *Pythium* control were not effective in any of the trials in North Carolina.

As mentioned previously, the QoI fungicides are not known for control of diseases caused by the Oomycota. In addition, multiple articles have suggested that applications of QoI’s increase plant yield and vigor (2). Therefore, it was hypothesized that the excellent control of *Pythium* root dysfunction by Pyraclostrobin was due to increased creeping bentgrass yield and vigor as well as detrimental effects of the fungicide on *Pythium volutum* growth. The objectives of the experiment that utilized the phytotron space were: 1. Determine the effects of pyraclostrobin on creeping bentgrass growth rate. 2. Evaluate *Pythium volutum* sensitivity *in vitro* to pyraclostrobin, azoxystrobin, and mefenoxam.

Materials and Methods:
Methods to Determine the Physiological Effects of Pyraclostrobin on Creeping Bentgrass.

- Creeping bentgrass (*Agrostis stolenifera*) was seeded into containers containing USGA sand.
- Plants grown in greenhouse for six weeks.
- After six weeks nitrogen treatments were initiated (0, 50, 100, 200 mg kg-1) and applied daily for four weeks.
• Pyraclostrobin was applied (0, 140, 280, 560, 1120, 2240, and 4480 g ha-1) using
a spray chamber.
• Treatments arranged in a CRD design with 6 replications.
• Clippings were collected every two weeks, dried, and weighed to assess foliar
growth rate.

Methods to Determine the Effect of Pyraclostrobin on *Pythium volutum* growth rate

• Sensitivity in culture was determined for five isolates that comprise three *Pythium
volutum* genotypes.
• Five concentrations (0, 0.001, 0.01, 0.1, 1, 10 mg kg⁻¹) of pyraclostrobin,
azoxystrobin, and mefenoxam were examined.
• Isolates were transferred to fungicide amended media using a 4 mm cork borer.
• Plates were incubated at 230°C for ~ 5 days.
• Colony diameter was measured and linear regression was used to estimate EC50
values

Results:

Pyraclostrobin did not increase creeping bentgrass yield at any rate and there was
not an interaction between nitrogen rate and pyraclostrobin rate (Table 1.). However,
*Pythium volutum* is extremely sensitive to Pyraclostrobin in culture, which may explain
the excellent control observed in field trials (Table 2). Yet, *P. volutum* is also highly
sensitive to Azoxystrobin and Mefenoxam. These chemicals were ineffective against
Pythium root dysfunction in the field.

Conclusions:

• Pyraclostrobin does not increase creeping bentgrass growth rate.
• *Pythium volutum* is highly sensitive to pyraclostrobin.
• Pyraclostrobin in vitro results help explain it’s effectiveness in fungicide trials.
• Variation among *P. volutum* genotypes may help explain variation of fungicide
efficacy observed in NC.
• Mefenoxam and azoxystrobin results do not explain the lack of control observed
in field trials.
• This data indicates the need to examine other fungal characteristics such as
zoospore formation and germination.

References:


2. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., and Parr-
58:649-662.
Table 1. The effects of nitrogen and Pyraclostrobin application rates on creeping bentgrass growth rate under growth chamber conditions. Growth rates are averaged over three collection dates. Means with the same lower case letter are not statistically different (LSD p=0.05).

<table>
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Table 2. The effects of Pyraclostrobin, Azoxystrobin, and Mefenoxam on *Pythium volutum* mycelial growth rate in culture. Values indicated as greater than 10 were not on the regression line. Means within each fungicide with the same lower case letter are not statistically different (LSD p=0.05).

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<td>0.156 b</td>
<td>&lt;10*</td>
<td>&lt;10*</td>
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</table>

This research was presented at the Agronomy meetings in Salt Lake City in 2005 by Jim Kerns. He won 1ˢᵗ place in the graduate student oral competition in the C-5 division.
COMPARISON OF DETECTION OF COLLETOTRICHUM SPP.
IN STRAWBERRY PETIOLES BY BIOASSAY AND REAL-TIME PCR

Rosemary Schwegel, & Frank Louws
Department of Plant Pathology

Introduction:

Strawberry anthracnose, caused by the fungal species Colletotrichum acutatum, C. fragariae, and C. gloeosporioides, is widely considered the most economically important disease of strawberry in North Carolina. The use of disease-free planting stock is among the most important management techniques for this disease. The implementation of this technique, however, is complicated by the fact that Colletotrichum often infects plants latently, producing symptoms only when conditions are conducive. Detection of latent infections is currently performed according to a bioassay protocol (Mertely and Legard 2000) which takes 5-7 days.

Real-time PCR differs from standard PCR in that the number of DNA amplicons produced during thermal cycling is measured during the cycling by fluorescence rather than after cycling by gel electrophoresis. The fact that the measurement is performed during cycling results in a faster protocol (same-day results) and enables more accurate quantification of the initial amount of DNA.

This project was designed to compare the performance of the bioassay to that of a newly developed real-time PCR protocol which has the potential to be both more rapid and more sensitive than the bioassay.

Materials and Methods:

In May 2005, ninety-six strawberry plug plants were obtained from the North Carolina State University Micropropagation Unit and installed in the NCSU Phytotron’s growth chambers. After one week, these were transplanted to six-inch pots in a 50% peat-lite, 50% sand potting medium. Twenty-four plants were placed in each of two 3m² walk-in growth chambers set at 26/22°C under 14 hours of fluorescent light to suppress flowering and encourage vegetative growth. The remaining forty-eight plants were transferred to a Phytotron greenhouse under the same temperature conditions with natural light plus three hours of supplemental lighting to increase the day length. All plants were watered once daily with the standard nutrient solution.
One 4 cm petiole sample was collected from each plant at four sampling times. The first petiole sampling was performed prior to inoculation, and additional samplings were conducted at 5, 12, and 19 days after inoculation. Scissors and forceps sterilized with 70% ethanol were used to collect the petioles, which were placed individually in 10 ml tubes. These petioles were sliced longitudinally with a sterile scalpel, one half being assigned to the bioassay and the other to the real-time PCR assay. The plants were spray-inoculated with a $10^6$ spores/ml solution of *Colletotrichum acutatum* grown from cultures obtained from the NCSU Plant Disease and Insect Clinic. After the last sampling, the first set of forty-eight plants was removed from the chambers. The chambers were sterilized, and the experiment was repeated with the remaining forty-eight plants from the greenhouse.

**Results and Discussion:**

The results from the comparison of the two assays may be seen in Figure 2, below. The number of positive results for the real-time assay was determined by calculating the initial DNA concentration according to the standard curve from each run. The initial concentration was calculated in terms of number of copies of the DNA template per PCR reaction tube. The cut-off point for a positive sample was set at 10 and 100 copies of template DNA per reaction to assess the ways in which the assay sensitivity was affected.
Figure 2: Comparison of detection of Colletotrichum infection in growth chamber-grown strawberry plants by bioassay and real-time PCR.

These results show that setting the cut-off for positive results at 10 or 100 copies per reaction can maximize sensitivity or minimize visibility of background contamination, respectively. Low levels of background contamination are visible in the two pre-inoculation samplings (Day 0) at both cut-offs, but are less visible at 100. The high rates of real-time detection and low rates of bioassay detection after inoculation indicate the sensitivity of the real-time assay. The decreasing level of infection in the second repetition reflects the observed low infection rate at that time.

Real-time PCR has the potential to replace the traditional bioassay for strawberry anthracnose, and is particularly useful in detecting latent infections. This assay may be used to screen mother plants, daughter plants and planting stock to avoid introducing the pathogen into propagative or fruiting fields, or may be used in research to track the spread of disease within a single plant or throughout a field.

Acknowledgment:

Support for this project was provided by the North Carolina State University and the NC Strawberry Growers Association.

Literature Cited:

Tobacco blue mold, caused by the oomycete pathogen *Peronospora tabacina* is a highly destructive pathogen of tobacco seed beds and production fields in North Carolina and worldwide. The disease has been responsible for multi-million dollar losses to growers and resistance to the commonly used fungicide metalaxyl has made management increasingly difficult. Blue mold can be extremely severe in tobacco transplant production houses and movement of infected plants to the field can lead to widespread disease occurrence. In previously funded work we developed a PCR primer called Ptab that can be used to specifically identify the blue mold pathogen in infected tissue (Fig. 1). We have also developed diagnostic PCR-RFLP (restriction fragment length polymorphisms) fingerprints that allow us to distinguish all the major tobacco leaf pathogens. A manuscript from this research is now under review for Phytopathology.

There are several different hypotheses about the dispersal of *P. tabacina* including:

a. The inoculum source for commercial winter tobacco in the US is in the tropical zones (south of the 30th parallel of latitude). It is also believed that the pathogen does not overwinter in the more temperate zones, and that inoculum is introduced anew each year into the U.S.A.

b. Inoculum of *P. tabacina* can overwinter in wild *Nicotiana* species (Texas) (pathogen undergoes sexual reproduction)

c. The pathogen overwinters in some regions of the U.S.A. Evidence to support this theory is that in 2004 some states such as Florida, Georgia and South Carolina were not affected by blue mold but states further North were affected by the disease. In addition, metalaxyl-insensitive isolates were found in the Caribbean (reports from Cuba) in 1981, but were not found in Florida until 1991. Others have reported that the pathogen can overwinter in transplant beds in greenhouses.

We plan to test these hypotheses by conducting population genetic studies using isolates collected from different geographical regions and years. It may be that a combination of the above events occur that lead to blue mold epidemics. We plan to sequence specific regions of the nuclear and mitochondrial genome of the pathogen. In the past year we have made considerable progress in developing additional primers that will be used for gene sequencing of specific mitochondrial, nuclear and ribosomal genes (Fig. 2). Isolates originating from the same source should have similar sequences if the genes are not under selection, while variable haplotypes would indicate multiple sources of inoculum or the possibility of genetic recombination.

We have done some preliminary restriction digestion of one of the amplified mitochondrial genes, *Cox 2* gene with *Alw I* (Fig. 3). No differences were observed among isolates and two fragments were observed. This does not indicate that there are no differences among isolates, since mutations can be generated by a transversion of a nucleotide, which could generate the same size fragment. We plan to start sequencing the
amplified genes, align the sequences and look for informative sites that may lead to differentiation of haplotypes among the isolates.

POTENTIAL SCIENTIFIC, ECONOMIC, AND OR SOCIAL IMPACTS

Specific strains of the blue mold pathogen in tobacco transplant beds could be directly tracked to field epidemics once we identify the mutations leading to haplotypes. We are also evaluating the fungicide sensitivity of some of the isolates of *P. tabacina* in collaboration with Kelly Ivors at the Mountain Horticultural Crops Research Station. This information will be useful for growers as they select fungicides to apply or varieties to plant in the field.

Our lab in the department of plant pathology at N.C. State maintains one of the largest preserved collections of the tobacco blue mold pathogen in the world. Charlie Main, Tom Melton, Paul Shoemaker, Kelly Ivors and county agents from many states have provided isolates from flu-cured and burley tobacco. The collection is a valuable resource as researchers, extension personnel and regulatory scientists develop improved methods to manage this important disease for NC tobacco growers. The cultures are maintained in cryostorage and on tobacco plantlets in tissue culture (Fig. 4). Last year we increased our collection from 175 to 204 samples. The collection includes isolates from the USA and international samples from Mexico and Dominican Republic, and western Europe. Our lab now houses the Ft Detrick International blue mold collection. Within USA, we have samples from North Carolina, Connecticut, Florida, Georgia, Kentucky, Maryland, Pennsylvania and Texas. At the end of this year we hope to obtain more samples from Dominican Republic and Mexico, and new ones from Costa Rica, Nicaragua, Honduras and Cuba. Maintaining and increasing the collection requires a lot of effort and time. Each sample will be inoculated onto an *in vitro* plant before analysis of DNA, to establish a clean and pure DNA sample in the Phytotron.

Fig. 1 PCR amplification of the ITS and 5.8 S rDNA with the PTAB/ITS 4 primers from *P. tabacina* samples retrieved from cryostorage. A series of isolates stored in cryostorage from 1979 to the present time were tested.
Fig. 2. Amplified genes from isolates of *P. tabacina* using A,) mitochondrial primers P3(*rpl5,rpl14, tRNA’s*), B, Cytochrome c oxidase subunit 1 gene (COX1), and C Cytochrome c oxidase subunit 2 gene (COX2) B) nuclear primers NADH dehydrogenase subunit 1 gene (*nadh1*), B- tubulin gene and part of Ras gene  C) ribosomal primers LR9/LR7-0 and LR3/LR16-0, parts of the Large subunit 28S
Fig 3. Digestion of mitochondrial Cox 2 gene with the Alw I restriction enzyme.

Fig 4. Induction of in vitro plantlets from seeds.  This process takes about two months from seed to plantlets.  This process has the advantage of generating aseptic plants.  The plantlets are then inoculated with isolates of *P. tabacina* and the isolates are maintained for subsequent DNA work in the close containers.
USING GRAFTING TO INDUCE DISEASE RESISTANCE AND INCREASED YIELDS IN HEIRLOOM TOMATO PRODUCTION SYSTEMS
Cary Rivard, & Dr. Frank Louws

The use of grafted tomatoes for commercial production has been implemented worldwide, where soilborne disease pressure is high. Grafting has been used to manage *Fusarium*, *Verticillium*, Root-knot nematodes, and bacterial wilt in several Asian, Mediterranean, and northern European countries. However, this technique is relatively unknown in the United States. With the increasing direct-marketing avenues available to small, sustainable farmers, demand for vine-ripened heirloom varieties has increased dramatically. These varieties are open-pollinated, and are typically very susceptible to an array of soil-borne and foliar diseases. A research program was initiated to investigate basic and applied components of tomato grafts as a tool to manage soilborne diseases and increase crop productivity. This report highlights the applied objective to investigate the potential of grafting as a management tactic to reduce bacterial wilt (*Ralstonia solanacearum*) disease incidence in heirloom tomato production systems.

During the summer of 2005, three field experiments were implemented in order to determine the efficacy of grafted heirloom tomatoes in naturally infested soils. CRA 66 and Hawaii 7996 rootstock lines were identified through an extensive literature search, and utilized in 2 of the 3 experiments. These lines have been used by breeders as a source of resistance, but have poor fruit quality. In the Pender County experiment, plants grafted onto resistant rootstock did not wilt and had significantly higher harvest yields than the non-grafted control plants. In contrast, 100% of the non-grafted and self-grafted plants were killed by *R. solanacearum*. The Rowan County field experiment had a low incidence of bacterial wilt but plant growth data was informative. CRA 66 improved root vigor and plant dry weight (*P=0.05*) compared to non-grafted plants and generated similar yields. Root vigor and plant dry weight for Hawaii 7996 and the self-grafted plant were intermediate. The Wayne County trial was non-replicated and designed to pursue questions about training and management, using ‘Maxifort’, bred specifically for rootstock use, within an organic production context. Training experience and crop productivity was informative. Grafted tomato plants reduced bacterial wilt incidence and
increased plant growth or tomato yield, even in the absence of disease pressure. Grafting could be a valuable tool for heirloom tomato farmers who wish to increase disease resistance without the use of chemical treatments.
EFFECT OF TEMPERATURE ON SOYBEAN FATTY ACID COMPOSITION AND PATHOGEN DEFENSE GENE EXPRESSION
Xue, HQ¹ and Upchurch, RG²,³

¹Crop Science Department, ²ARS Soybean & Nitrogen Fixation Unit, ³Plant Pathology Department, NC State University, Raleigh, NC

The project began on October 13, 2005 when pre-germinated seed of three near isogenic soybean lines differing in oleic acid content were planted. Seed of the near isogenic lines N303-3, N303-4, and N303-7, when grown in the field, yielded respectively, 57.4, 45.0, and 35.7 percent oleic acid. These soybean lines were developed by J.W. Burton of the USDA-ARS Soybean & Nitrogen Fixation Unit at NC State University. Plants in each of three B-type chambers were arranged in a randomized complete block design. The experiment concluded with the final harvest of mature seed on January 24, 2006.

Initially, all three chambers were set for a 26/22 °C with a 12 hr day. When seed pods had developed to R5 stage (pods near full length, but seeds 4mm and flat) the temperature setting of two of the chambers was changed to 22/18 °C or 34/26 °C, each maintaining the 12 hr day. Thus, seed development of the three lines continued to maturity in three different temperature environments. Seeds were harvested at 35 days after flowering (DAF) and at maturity for experiments. The research objectives were the following:

1) Determine what affect the different temperatures had on oleic/linoleic acid content of developing seeds 35 DAF and seeds at maturity.
2) Assess the susceptibility of seeds with significantly different oleic/linoleic content at 35 DAF and at maturity to colonization by the fungal pathogens Cercospora kikuchii and Diaporthe phaseolorum using fungal ergosterol as a marker of fungal biomass.
3) Characterize the expression of defense genes PR-1 and PDF1.2 in soybean seed with significantly different oleic/linoleic content at 35 DAF following induction by inoculation with the two fungal pathogens or treatment with salicylic and jasmonic acids.

Analysis of fatty acid composition of seed at 35 DAF has been completed and shows that temperature dramatically affects oleic/linoleic acid content of seed at this stage of development in these lines. Pathogen infection and chemical treatments of seed 35 DAF have been completed and the seed are frozen and awaiting analysis of seed colonization and defense gene expression. Mature seed chips have been collected for fatty acid analysis. Pathogen inoculation of mature seed will begin shortly.
THE INFLUENCE OF INCREASING P SUPPLY ON SEED P COMPOSITION OF SOYBEAN LINES WITH INDIVIDUAL OR BOTH RECESSIVE GENES THAT CONDITION THE LOW SEED PHYTIC ACID TRAIT

Dan Israel, Prachuab Kwanyuen, Joe Burton and Ralph Dewey

Introduction

Wilcox et al (2000) have developed a soybean mutant with seed phytic acid-P concentrations that are 80% lower than normal. Genes from this material have been crossed into germplasm adapted to several regions of the U.S. Inheritance studies associated with these breeding programs have shown that the low seed phytic acid phenotype derived from the mutant of Wilcox et al (2000) is conditioned by two recessive genes designated as \(pha1\) and \(pha2\) (Oltmans et al. 2004). The enzymatic steps in the biosynthetic pathway that have been altered in this mutant to generate the low phytic acid phenotype have not been identified.

Unique genetic material has been developed in the breeding program of Dr. Roger Boerma at the University Georgia for study of the low seed phytic acid trait in soybean. Our objective was to evaluate the influence of P supply from deficient to excessive levels on seed phytic acid P and phosphate P concentrations in four of these genotypes. A normal seed phytic acid genotype (Prichard-RR), a low seed phytic acid genotype (G03PHY-443 derived from crossing Prichard-RR with CX1834-1-2 and 3 backcrosses of low phytic acid progeny with Prichard-RR as recurrent parent), a genotype with \(pha/pha\) alleles at the Satt561 locus on linkage group L and \(Pha/Pha\) alleles at the Satt237 locus on linkage group N (LGL) and a genotype with \(Pha/Pha\) alleles at Satt561 locus on linkage L and \(pha/pha\) alleles at the Satt237 locus on linkage group N (LGN) were supplied with deficient to above optimal P levels.

Methods

Plants were grown in A chambers with 600 micromols.m\(^{-2}\).s\(^{-1}\) of photosynthetically active radiation and 400 \(\mu\)L.L\(^{-1}\) CO\(_2\). A day:night temperature of 26:22 C was used throughout the experimental period and a 9 hour photosynthetic period followed by a 15 hour dark period with a 3 hour interruption with photomorphogenic irradiance in the middle of the dark phase was used until the 2\(^{nd}\) trifoliolate leaf began to unroll. At this stage the night interruption was discontinued to induce flowering.

Three seeds of soybean lines described above were planted into thoroughly moistened peatlite-gravel mix in 10 inch pots and placed into the controlled environment chambers. After emergence pots will be thinned to one healthy seedling. Plants were supplied Phytotron nutrient solution modified to contain deficient (0.05mM), optimal (0.5 mM) or supra-optimal (0.9 mM) P. The choice of P levels was based on previous work with soybean in greenhouse and outdoor pot culture. Three replicates of each genotype by P level treatment were used. Treatments were arranged in a randomized block design with 3
blocks (2 blocks in one chamber and 1 block in a second chamber set for same environmental conditions).

At harvest maturity pod number, seed number and seed dry mass were determined. A sub sample of each seed sample was ground to pass a 1 mm screen for measurement of phosphate-P, phytic acid P, protein and oil concentrations. Total P was measured by inductively coupled plasma emission spectroscopy; phosphate-P was measured by a modification of the microtiter plate assay of Larsen et al (2000); phytic acid-P was measured by anion exchange HPLC after extraction with 0.5 N HCl (Kwanyuen and Burton, 2005); total protein was measured by the Dumas reductive combustion method coupled with thermal conductivity detection; and oil was measured by pulsed NMR.

**Results and Discussion**

Seed yield increased between the 0.05 mM and 0.5 mM P levels and either remained the same or decreased at the 0.9 mM P level for all genotypes (Table 1). The negative influence of the 0.9 mM P treatment on seed yield was greatest for the G03PHY-443 line which has low seed phytic acid (Figure 1). This decrease in yield was primarily associated with a decrease in seed size (i.e. 100 seed weight, Table 1). These yield results confirm that P treatments ranged from deficient to excessive levels.

**Table 1. Influence of P supply and soybean genotype on seed yield and yield components**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nutrient solution P conc.</th>
<th>Seed dry weight g/plant</th>
<th>Seed number per plant</th>
<th>Pod number per plant</th>
<th>Seeds per pod</th>
<th>Weight per 100 seed grams</th>
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<tbody>
<tr>
<td>PrichardRR</td>
<td>0.05</td>
<td>27.85</td>
<td>157</td>
<td>76</td>
<td>2.06</td>
<td>17.7</td>
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<tr>
<td></td>
<td>(1.11)&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>(9)</td>
<td>(2)</td>
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<tr>
<td></td>
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<td>236</td>
<td>113</td>
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<tr>
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<td>247</td>
<td>116</td>
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<td>(130)</td>
<td>(54)</td>
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<td>G03PHY-443</td>
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<td>48</td>
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The normal phytic acid line (Prichard-RR) and the genotypes with homozygous recessive \((pha/pha)\) alleles at one of two loci governing the low phytic acid trait (LGL and LGN) exhibited almost identical increases in seed phytic acid concentration (2.0 to 7.0 mg phytic acid-P/g dry wt.) in response to increased external P supply. The phosphate-P concentrations in seed of these genotypes were low (0.2 to 0.6 mg/g dry wt.) and increased very little in response to increased P supply. In contrast, seed of the G03PHY-443 line which is homozygous recessive \((pha/pha)\) at both loci controlling the low phytic acid trait had 2.0 mg phytic acid-P/g dry wt. in seed and this did not increase as the external P supply was increased from deficient to above optimum levels. The phosphate-P concentration in seed of this line increased 5 fold as external P supply was increased from deficient to above optimum levels.

**Figure 1. Influence of P supply on the seed P composition of soybean lines with different combinations of genes required for low seed phytic acid composition**
These results clearly demonstrate that alterations at two loci are obligatory for prevention of increases in seed phytic acid concentration in response to increased P supply. These results do not support our hypothesis that alleles at one of the two loci may encode a P inducible enzyme in the phytic acid biosynthetic pathway. The results provide additional support for conclusions from inheritance studies that the low phytic acid trait in lines derived from line CX1834 is conditioned by two recessive genes.

The P composition had no influence on the total protein and oil concentrations in the seed (Table 2). Protein concentration decreased and oil concentration increased as P supply was increased (Table 2). However, protein concentration remained above 41% and oil concentration increased to about 20% for all treatments. These results indicate that changes in P composition from predominately phytic acid to predominately phosphate did not alter the balance between protein and oil synthesis during seed development.

Table 2. Influence of P supply and soybean genotype on seed protein and oil concentration

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<tr>
<td></td>
<td>mM</td>
<td>%</td>
<td>%</td>
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<td>0.9</td>
<td>41.5</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>(1.2)</td>
<td></td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

†Values in parenthesis are standard deviations.
Acknowledgements

We appreciate the support of the Phytotron staff in maintaining growth chambers and the United Soybean Board for providing partial funding to support the research.

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ASSESSMENT OF RATE OF HERBIVORY ON NEWLY FLUSHED VERSUS MATURE FOLIAGE.

Harold Heatwole
Dept. of Zoology

During 2005, I made use of the computerized planimeter of the Phytotron to measure the extent of grazing by insects on leaves of various species of trees in Madagascaran rainforest, and on several species of Eucalyptus introduced from Australia into various countries. In total, *** leaves of *** species of trees were measured.

The technique was to collect samples of leaves and in the field to trace their whole outlines on paper, then to trace the outlines of parts that had been eaten by insects, separately by grazers and by skeletonizers. Upon returning to the United States, I first cut out the outlines of the leaves with manicure scissors and ran the paper cutouts through the planimeter and got the surface area of each individual leaf. Then I cut out the parts that represented damage by insects and measured each leaf again. The difference between the two measurements for a given leaf gave the area that had been consumed. For analysis I expressed the damage in terms of percentage of the leaf consumed. I also summarized the incidence of attack (percentage of leaves that had been attacked).

Madagascar project: In Madagascar I compared the extent of herbivory on leaves at different levels of the forest, from ground vegetation, through shrubs into the first canopy later, the top canopy and into the scattered emergent trees. Sampling was also conducted on different sides of emergent trees (windward and leeward), within the canopy, and the epiphytes, parasites and lianes attached to the trees. Sampling was achieved by rope climbing techniques, from stationary tree houses, a canopy raft and from powered hot-air dirigible and from an individual harness helium balloon. This project will allow assessment of the spatial variation of herbivory within the forest. Sequential sampling of new growth allowed assessment of rate of herbivory on newly flushed versus mature foliage.
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Physiological and Biochemical Responses to Ozone

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Abit, P. & W. Hoffmann

Abit, P. & W. Hoffmann

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Assessment of Soybean Root Growth in Compacted Soil

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Phenotypic Plasticity of Soybean in Response to Nitrogen Deficiency

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The Effect of Frugivory By Anolis Curvieri on The Seed Germination Of Three Plant Species
The Southeastern Plant Environment Laboratory, often referred to as the North Carolina State University Phytotron, is especially designed for research dealing with the response of plants, small animals, and microorganisms to their environment. A high degree of environmental control makes possible simulation of a wide range of climates found in tropical, temperate and northern zones.

Research in the Phytotron deals with all phases of plant biology. Although the majority of the studies are conducted with agricultural crop species, the Phytotron can accommodate ecological investigations, plant biology problems of the space program, experimental taxonomy and air pollution studies as well as basic physiological and biochemical research.

The Phytotron facility is available to the resident research staff, participants in graduate research programs of North Carolina State University and to domestic and foreign visiting scientists.