PHYTOTRON REPORT 2006

NC STATE UNIVERSITY

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Cover: Dwarf Dogwood *(Cornus cadensis)*

NCSU Phytotron

A Controlled Environment Facility

Annual Report 2006

North Carolina State University

College of Agriculture and Life Sciences

Tomorrow's Science And Technology...Today

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Carole H. Saravitz

Usage by Chamber

Usage for all growth chambers in 2006 was 102% of the recommended optimal occupancy, or 84% of maximal occupancy (Table 1). The 17 individually programmed A-chambers were occupied at 147% of optimal capacity and the five "standard" A-chambers had a 78% optimal occupancy rate. For 2006, total A-chamber usage was 131% the recommended optimal occupancy. Usage of B-chambers was at 82% and C-chambers, 87 % for the year.

During 2006 the glasshouses had an optimal occupancy rate of 34%. A large portion of the glasshouse space allocation was utilized for studies with student projects (Bio 183), corn (Sederhoff; Plant Biology), turfgrass (Dong & Qu, Crop Science), cotton (Haigler, Crop Science), tomatoes (Sederoff & Khodakovskaya; Plant Biology),

The two walk-in rooms equipped with high intensity discharge lamps (HID) were in use during 70% of the year for studies examining the growth responses of soybean (Israel, Soil Science;).

Usage by Department

83 different projects were conducted in the Phytotron during 2006 by faculty and students from 9 departments in the Colleges of Agriculture and Life Sciences (Table 2). The Crop Science Department used the largest amount of space in 2006, nearly 35 %, for 24 different projects. Secondly, the Plant Biology Department used more than 19% of the space for 18 projects. The Plant Pathology Department used nearly 16% of the space for 16 projects, and Horticultural Science used over 6% for 9 projects and Entomology used 6% for three projects. Genetics had a space use allocation of approximately 5%. Microbiology and Forest Resources each used 1% and 3% was used by Biological Sciences for the BIO 183 class for student projects.

Usage by Crop Type

15% of the space used in the Phytotron during 2006 was used to grow arabidopsis while 13% was allocated for growing soybean (Table 3). Research with other agronomic crops included corn (12%),) and tobacco (13%). Space for research on vegetable crops used 10% of

the space in 2006, weeds 4%, ornamentals, 2% and for trees, 5%. Space for research on turfgrass used , cotton, 2% and 3% of space was used for growing rice. The 'Demonstration' category (2%) included space for plants grown for display during tours of the facility.

Phytotron Visitors

A portion of our outreach and educational goals includes offering tours of the Phytotron. During the year laboratory sections from NCSU plant biology, crop science, and horticultural science classes; Meredith College; and numerous high school and middle school 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 the Netherlands, Moldova and Republic of Congo.

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, high intensity light chambers, glasshouses, and plant pathology. 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 Plant Biology and Crop Science.

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.

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[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, 2006**

Chamber*		**% Optimal**	**% Maximum**
A-Chambers	(17 Individual)	147	92
A-Chambers	(5 Standard)	78	49
A-Chambers	(22)	131	82
B-Chambers	(10)	82	82
C-Chambers	(22)	87	87
Glasshouses	(5)	34	27
HID Walk-In	(2)	70	70
Tall Chamber	(1)	48	44

* Dimensions of Chambers are:
A = 8' x 12' x 7'h
B = 8' x 4' x 7'h
C = 4' x 3' x 4'h
H = 10' x 6' x 8'h
T = 16' x 12' x 7'-15'h

Utilization of all growth chambers during 2006:
Optimal Usage = 102
% Maximal Usage = 84

Table 2. **DEPARTMENT USAGE SUMMARY, 2006**

Department	**% Total Use-Days**	**# Projects**
Crop Science	35	24
Entomology	6	3
Forest Resources	1	1
Genetics	5	3
Horticultural Science	6	9
Microbiology	1	2
Phytotron	7	4
Plant Biology	19	18
Plant Pathology	16	16
Soil Science	1	1
Teaching	3	2

*83 Studies Conducted in the Phytotron During 2006

Table 3. **CROP TYPE SUMMARY, 2006**

Crop	**% Total Use-Days**
Arabidopsis	15
Corn	12
Cotton	2
Demonstration[a]	2
Grains[b]	3
Insect[c]	1
Maintenance	2
Ornamentals[d]	2
Other[e]	8
Rice	3
Soybean	7
Tobacco	13
Trees[f]	5
Turfgrass[g]	11
Vegetables[h]	10
Weeds[i]	4

Includes:
[a]Celosia, Corn, Himalayan Barley, Marigolds, Mung Beans, Peas, Pigweed.
[b]Wheat, Rye
[c]Argentine ants, Honey Bees
[d] Dogwood, Geranium, Helleborus, *Setcreasia purpurea*, Rhododendron
[e]Clover, *Lotus japonicus*, *Medicago truncatula*,
[f] Aspen, Fraser Fir, Oak,
[g] Bentgrass (*Agrostis palustris*), Bermudagrass, Saint Augustinegrass, and Tall Fescue
[h] Cucumber, Potato, Tomato, Watermelon
[i]*Commelina benghalensis,* Japanese Stiltgrass

The Puzzling Role Of Peroxidases In Plant Responses To Ozone – An Investigation Using *Arabidopsis Thaliana*

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Introduction

One of the early events following an acute ozone (O_3) exposure in sensitive plants is a bimodal oxidative burst (Kangasjarvi et al., 2005; Schraudner et al., 1998). The apoplastic reactive oxygen species (e.g., $O_2^{\cdot}$-, and H_2O_2) generated in these bursts are suggested both to induce damage and act as signaling molecules that elicit plant defense responses (Kangasjarvi et al., 2005). Suppression of the potentially damaging oxidative bursts putatively occurs through several mechanisms including antioxidants such as ascorbate (Burkey and Eason, 2002) and enzymatic reduction via superoxide dismutase and peroxidases (Kangasjärvi et al., 1994). Increased peroxidase activity has been reported following both acute and chronic exposure to O_3 in a number of species (Burkey et al., 2000; Peters et al., 1989; Ranieri et al., 2003), including *Arabidopsis* (Morgan et al., 2005). Reduction by peroxidases of H_2O_2 may be a mechanism for lowering H_2O_2 levels due to O_3 exposure, although generation of H_2O_2 by peroxidases may also occur (Ranieri et al., 2003). A definitive explanation for increased peroxidase activity in O_3-treated plants has eluded researchers for more than 30 years.

The classical plant peroxidases (class III) are targeted via the endoplasmic reticulum to the cell apoplast or the vacuole, and are considered to have a variety of functions, including lignification, suberization, auxin catabolism, defense, stress and developmentally related processes (Penel et al., 1992). There are 73 class III peroxidase genes in *Arabidopsis thaliana*, of which 58 are transcribed (Welinder et al., 2002). A Northern blot assay indicated that mRNA levels for peroxidase increased after acute exposure of *Arabidopsis* to O_3 (Sharma and Davis, 1994), although gene expression array experiments have not found altered regulation of peroxidase

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genes in O_3-treated plants (Li et al., 2006; Tosti et al., 2006). Also, there is no simple correlation between gene transcription and translation into protein. Plant protein extracts show only a few bands with peroxidase activity on native gels (Rao et al., 1997; Welinder et al., 2002). It is plausible, however, that peroxidases are activated by stress (Welinder et al., 2002).

To investigate a possible link between O_3 exposure and stimulated peroxidase activity, a study was conducted to determine whether infiltration of H_2O_2 into *Arabidopsis* leaves affected peroxidase activity. Also, we capitalized on the ready availability of peroxidase knockout mutants for *Arabidopsis* to examine whether peroxidase activity in O_3-treated plants was modified in the selected knockouts, thus providing information on which peroxidase genes might be involved in the response. The selection of genome-representative knockouts mutants was based on a clade analysis of *Arabidopsis* peroxidase genes.

Methods

Plant propagation

Homozygous knockout mutants were surface sterilized using 5 minute washes in 70% EtOH with 0.005% Triton X; 95% EtOH with 0.005% Triton X and 95% EtOH. Seeds were placed onto sterile Petri-dishes containing 0.5% PhytoAgar (Research Product International Corp., Mt. Prospect, IL) supplemented with 1% sucrose and ½ Murashige and Skoog with Gamborg's vitamins Media and stratified for 3 days at 4^o C. Seedlings were transplanted to Metromix200 in 5 x 5 x 5 cm cell-pack pots at 9 to 12 days of growth. Plants were grown for 5 weeks in 8 hr, 250 μmol m^{-2} s^{-1} light/16hr dark at 22-23^o C in the NC State University Phytotron.

The tDNA insertion lines of selected peroxidase genes were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH, USA). Homozygous lines were screened using PCR-based genotyping for the following insertion lines: Salk08082 (*per*14-1), Salk139401 (*per*14-2), and Salk127000 (*per*14-3), all within exons of gene located at locus At2G18140; CS804116 (*per*20-1) At2G35380; Salk014421 (*per*49-1) and Salk074490 (*per*49-2); and Salk057660 (*per*72-1). Gene specific primers for *per*14-1, *per*14-2 and *per*14-3 were: 5'-

CACAACCATAAATTTTTGCGC -3' and 5'- AAAAATTTCAGGGAGCCACAC -3'. Gene primers for *per*20-1 were: 5'-CGGAGCAAACCAATTCATTCCAGC-3' and 5'-CTTCGGAACAAAACAGAGTCCTTC-3'. The gene primers for *per*49-1 were: 5'-CATCTGCTTTGTCCCTCTCTG-3'and 5'AGTAGCTGTTGTCGAAGCTCG-3'; and for *per*49-2: 5'-CACTGACCTTGTCGCTCTCTC-3' and 5'-TAATTGAACGGAACTTGAGCG-3'. The primers for *per*72-1 were: 5'-ATCCTTAGATGCAATCCGGAC-3' and 5'-TCGATCTTGTCTCCCTCTCAG-3'. F2 plants were rechecked to confirm homozygosity of the inserts.

Experimental treatments

To test the role of H_2O_2 in inducing peroxidase activity, Col-O wild-type plants were selected from 5-week old sets. Leaves were cut at the petiole and placed immediately into Petri-dishes containing H_2O_2 solutions. Leaves from 5 plants (pooled) were submerged in the solutions with a nylon mesh and placed under 85 kPa vacuum for 3 minutes to infiltrate the apoplast with liquid. Following infiltration leaves were again floated on the surface of the solution to ensure gas-exchange. All samples were then placed in low light for 5 h. Afterward, the leaves were removed, blotted dry, frozen in liquid N_2, and stored at -80º C until assayed for peroxidase activity.

On the afternoon preceding an O_3 exposure, knockout plants were transferred to four continuous-stirred tank reactor (CSTR) chambers located in the North Carolina State University Phytotron, watered and allowed to acclimate overnight (~16 h). The CSTRs are Teflon-covered, cylindrical chambers that blend charcoal-filtered (virtually ozone-free) air with supplemental O_3 generated via electrical discharge through dry oxygen (model GTC-1A, Ozonia North America, Elmwood Park, NJ, USA). Ozone concentrations were either charcoal-filtered (CF) air or CF air plus 125 nmol $mole^{-1}$ 7 h d^{-1}. Ozone concentrations were maintained using a computerized feedback control system and mass-flow controllers. Following the two-d O_3 treatment, fully-expanded, mature leaves (mid-whorl) that were free of any visible injury were sampled. Distal portions of five leaves from one plant (100 mg) were frozen in liquid N and kept at -80^{o} C until assayed.

Peroxidase assay

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-µl aliquots were assayed spectrophotometically for peroxidase activity in a 2 ml volume containing 50 mM Na acetate buffer (pH 5), 0.5 mM H_2O_2 and 0.5 mM of 2,7-diaminofluorene (Criquet et al., 2001). Protein was determined using the BioRad Reagent and BSA. The experiment was conducted three times with two replicate chambers per treatment on each occasion.

All measured parameters are the average for three independent sets of plants separated in time measured in completely randomized design (n=2). Results were analyzed using mixed model analysis (SAS9.1) with treatment, genotype and interaction as fixed effects and plant set and associated interactions as random effects. To meet normality constraints, some data were ln transformed prior to analysis. Reported values are the LSMEANS and associated standard error of the LSMEANS. Significant differences are from *a priori* pairwise, linear contrasts ($\alpha = 0.05$).

Results and Discussion

It has often been noted that the increase in peroxidase activity following O_3-stress is in response to the reaction products of O_3, such as H_2O_2. To test if peroxidase activity increased in response to H_2O_2, leaves were infiltrated with H_2O_2 and assayed for peroxidase activity.

We found that additional H_2O_2 did not increase total peroxidase activity (Fig. 1). In fact, there was a significant decrease in peroxidase activity at the two highest H_2O_2 concentrations employed compared to the deionized water control. This decrease may be in response to losses in cell integrity and leaf turgor caused by the higher concentrations, especially at 10 mM H_2O_2, which resulted in substantial wilting of the leaves.

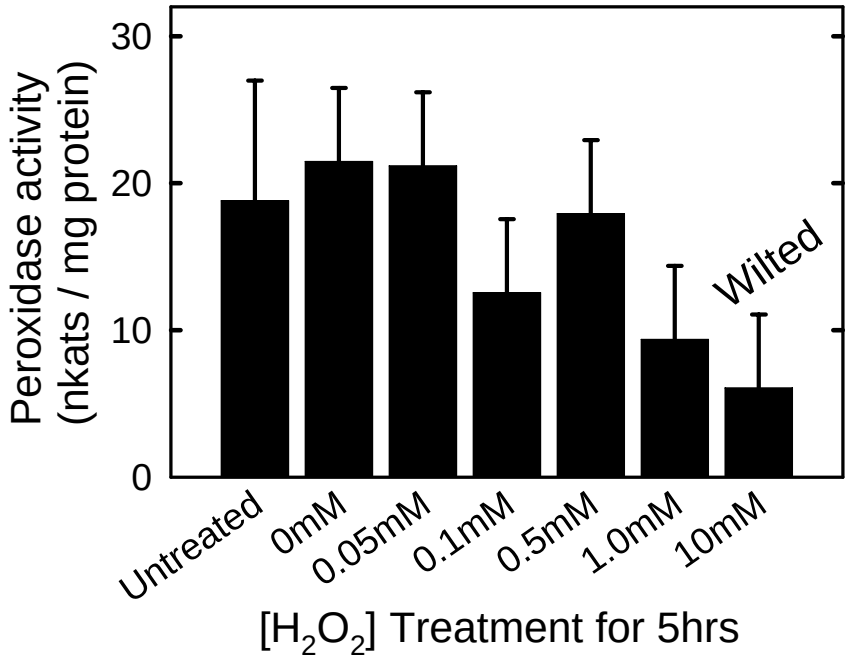

Fig. 1. Total peroxidase activity of *Arabidopsis* leaves vacuum-infiltrated with H_2O_2 solutions. Following infiltration, samples were floated on treatment solutions for 5 h. Bars are the LSMEANS of three replicate experiments of five leaves (pooled) in each experiment and the error bars are the standard error of the LSMEANS.

Peroxidase activity in four peroxidase knockout mutants treated with O_3 was also determined (Fig. 2). The stimulation in peroxidase activity following a two-day exposure to 125 nmol O_3 mol^{-1} was not controlled by any of the single genes selected. The knockouts all showed some increase in peroxidase activity in response to O_3, despite only three demonstrating a significant increase in activity. There was an indication that per20-1 and per72-1 may have increased baseline activity. None of the knockouts displayed visible injury symptoms due to O_3 that were atypical of Col-O (data not shown).

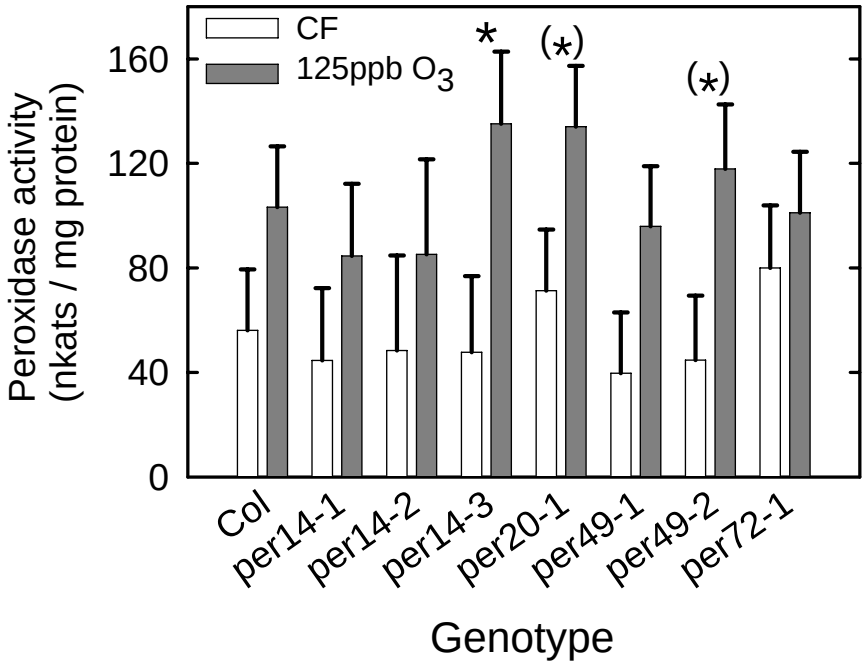

Fig. 2. Total peroxidase activity in peroxidase knockout mutants following two 8 h fumigations with CF air (open bar) or air enriched to 125 nmol O_3 mol^{-1} (grey-bar). Genes expression was interrupted by Salk standard t-DNA insertions with confirmed homozygosity. Values are the LSMEANS and associated standard error of the LSMEANS and linear pair-wise comparisons with each genotype are denoted with stars (no star = n.s.; (*) = $0.05<p<0.1$; * = $0.01<p<0.05$).

It was unexpected that infiltration of H_2O_2 into leaves did not stimulate peroxidase activity given previous studies of H_2O_2-induced stimulation of peroxidase activity and our current understanding of O_3 reactions in the leaf apoplast. For example, total peroxidase activity in tobacco leaf tissues was elevated one day after 0.5 mM H_2O_2 was applied as a foliar spray (Gechev et al., 2002). However, peroxidase activity was not stimulated in maize seedlings after roots were treated with 1 mM H_2O_2 in a nutrient solution (Azevedo Neto et al., 2005). Guaiacol peroxidase activity was not stimulated by 10 mM H_2O_2 administered through the petiole of cut *Arabidopsis* leaves, although activity of coniferyl alcohol peroxidase increased 60% (Rao et al., 1997). Salicylic acid also stimulated coniferyl alcohol peroxidase activity in a dose-dependent manner (Rao et al., 1997).

The numerous peroxidase genes in *Arabidopsis*, along with the low translation rate of peroxidase transcripts into protein, suggests that explorations of peroxidase function using knockout mutants

is an inefficient approach to identifying genes involved in O_3 responses until the genetic identity of the translated peroxidase isozymes is confirmed.

Altered ion regulation of the cell may be involved in the peroxidase response to O_3. There is evidence that peroxidase activity can be stimulated by Ca^{+2} (Penel, 1986), and it has been suggested that O_3 promotes Ca^{+2} influx in leaves (Peters et al., 1989). Experiments are in progress to test the effect of Ca^{+2} uptake alone and in combination with H_2O_2 and peroxidase substrates.

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Evaluation Of Possible Growth Effects Of Chemical Application On Turfgrass Under 3 Temperature Regimes And 2 Nitrogen Regimes

Danesha Seth Carley and Thomas Rufty
Crop Science Department

Introduction:

With the hot, humid summer conditions in the southeastern United States, heat stress is one of the major factors damaging cool-season bentgrass on golf greens. When temperatures increase, root and shoot growth decrease, contributing to what is commonly called summer bentgrass decline (SBD). Other factors associated with this decline include poor soil aeration, excessive or deficient soil moisture, and turf diseases. Although numerous strategies have been attempted in the field to minimize SBD during summer months, evidence is almost entirely anecdotal. One line of thinking is that the ability of bentgrass to withstand high temperatures is enhanced by higher nitrogen nutrition. Other observations suggest that frequent applications of chemicals promote general bentgrass health, perhaps by suppressing pathogen populations or by direct growth regulator effects. Further observations suggest that grass quality ("greenness", density, texture, etc.) is improved after repeated applications of specific chemicals immediately preceding and during periods of heat stress. Theoretically, the addition of certain chemicals might provide protection against UV light and increase the plant's antioxidant defense systems (Ervin et al., 2004a; Ervin et al., 2004b; Zhang et al., 2005).

The purpose of this project is to investigate the physiological basis for damage experienced by creeping bentgrass at high temperatures and to evaluate the efficacy of various treatments to minimize the high temperature stress damage.

M/M for study:

Established sod was planted in conetainers in Greens mix (90% sand and 10% peat). Plants were then grown at 3 different temperature regimes (22/18, 32/28, and 36/32 C), and 2 different nitrogen regimes (low and high). Then plants were spray treated with a variety of chemical treatments to see if any of these chemicals helped the plants better survive under stressful

conditions. Plants were harvested once or twice a week, as needed, and samples of leaf tissue were taken to establish leaf chlorophyll content, % carbon, and % nitrogen content. Visual ratings were taken weekly and at the end of the study, and root tissues were sampled to determine root weight and mass.

Results and Discussion:

Some of the chemical treatments did have an effect on growth and quality of the plants. Examples shown in Figs. 1 and 2 indicate that the chemical treatments affected the nitrogen concentration in the leaf tissues and visual quality. The responses occurred at optimal temperature and when the turfgrass was exposed to extreme heat. We were surprised to find that most of the positive effects occurred under the low N regime. We are currently conducting experiments which involve a series of nitrogen treatments to more precisely define the temperature/nitrogen response.

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Figures:

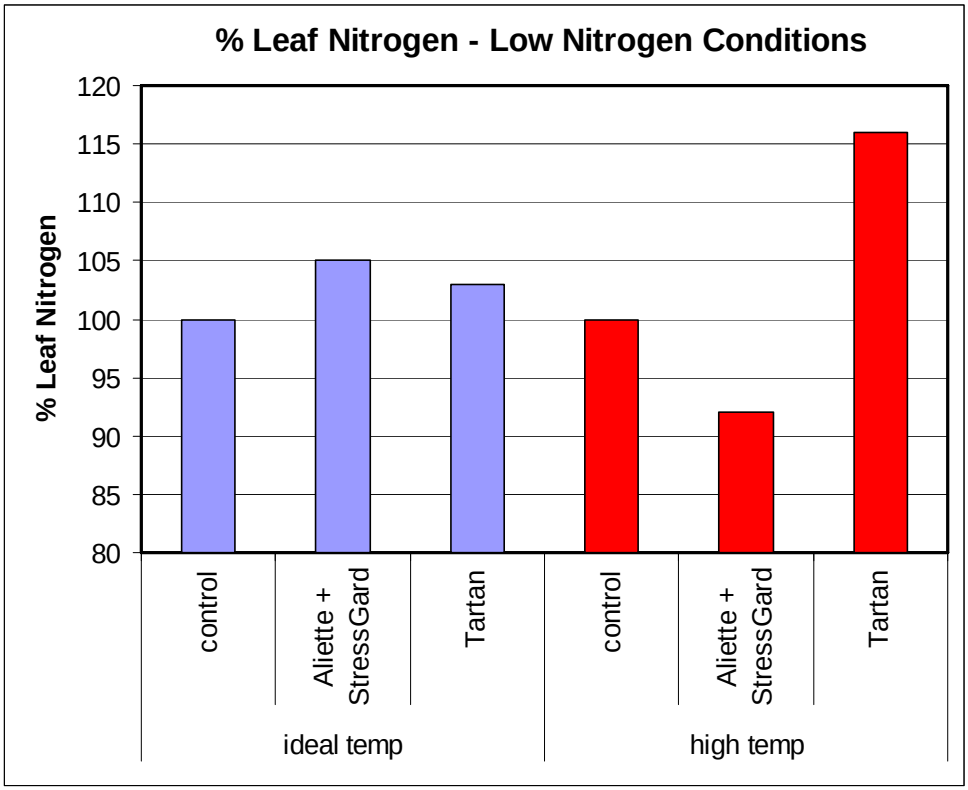

Figure 1. Percent leaf nitrogen for Aliette + StressGard treated plants and Tartan treated plants - as a percent of control - at ideal temperatures (22/18 C) and at high temperatures (36/28 C).

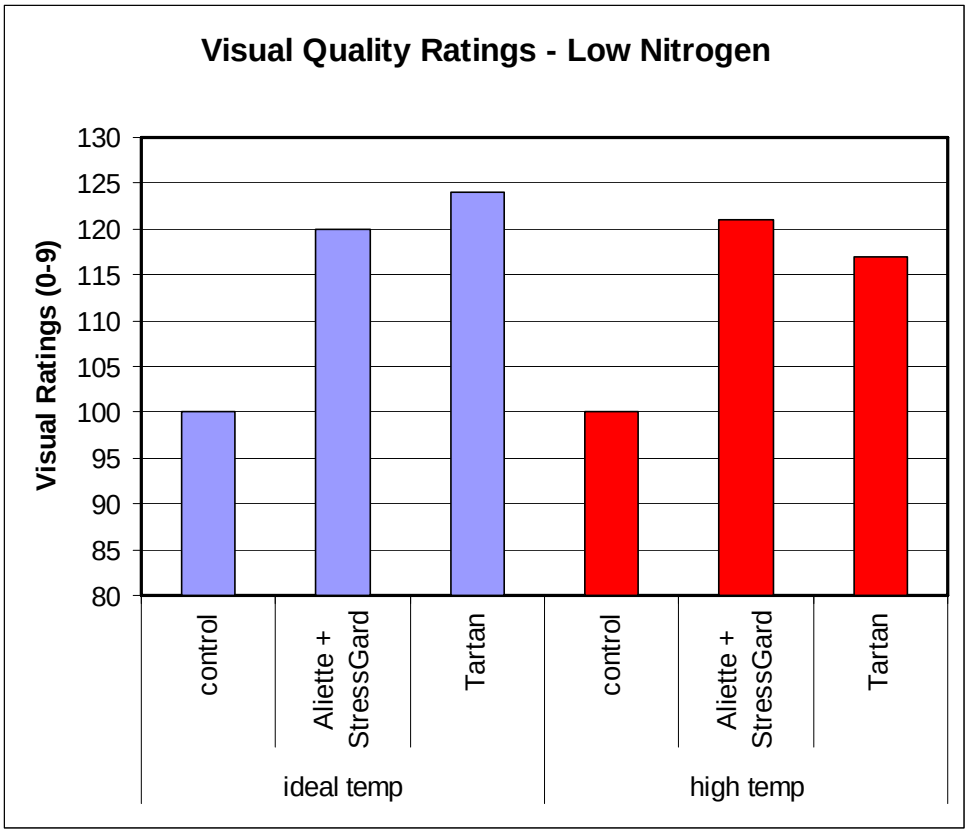

Figure 2. Visual ratings for Aliette + StressGard and Tartan treated plants as a percent of control- at ideal (22/18 C) and high temperatures (36/28 C).

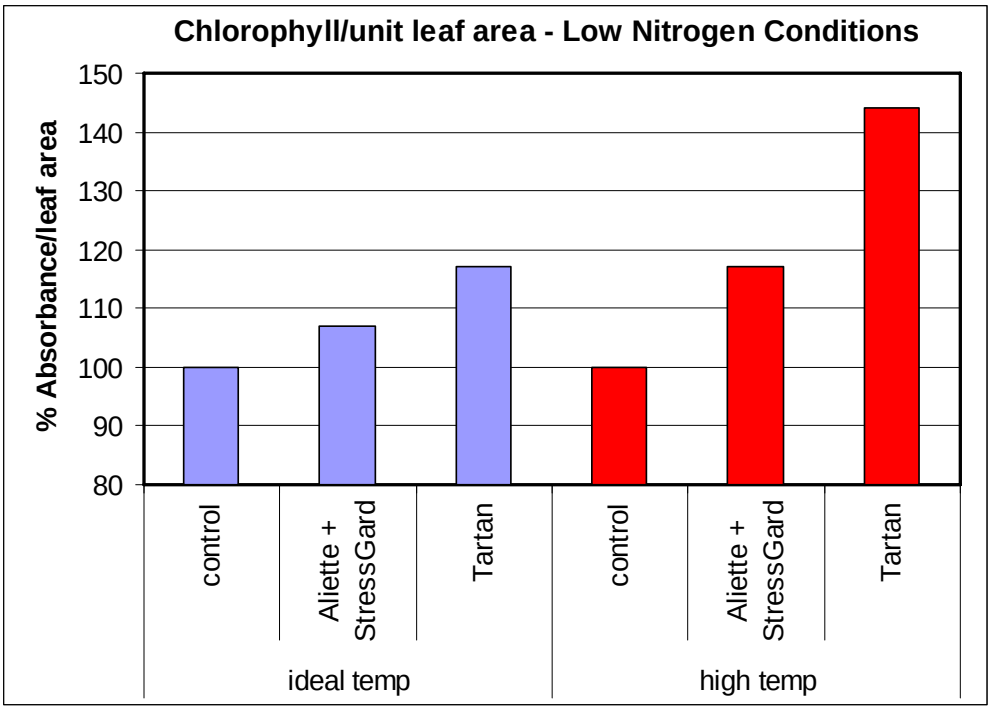

Figure 3. Chlorophyll, reported as % absorbance per unit leaf area for Aliette + StressGard treated plants and Tartan treated plants -as a percent of control- at ideal temperatures (22/18 C) and at high temperatures (36/28 C).

Variation In Response To Temperature Among Modern Maize, Mexican Landraces And Teosinte

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Studies were undertaken to asses the genetic variation among Zea species, i.e. Mexican maize landraces and related teosintes. The objectives of the research were to help define climatic requirements of current phytogenetic resources and to identify possible genetic variation for breeding programs. As the research is ongoing, this report will report on the role of temperature on the vegetative development of the various zea species and landraces.

Vegetative development in grasses characterized by the regular initiation and appearance of successive leaves at the stem apex. The rate of leaf initiation on the apical meristem and the rate of leaf appearance above the whorl (pseudostem) are largely controlled by temperature such that at constant temperature this rate is linear. Leaf development ceases when the stem apical meristem initiates reproductive primordia such that time to flowering can be described as a function of the number of leaves produced and the rate at which they are produced.

Studies including six Mexican maize landraces (Conico, Apachito, Tabloncillo, Tuxpeno, Chapalote, Zapalote Chico and Comiteco), a modern U.S.

maize hybrid (B73xMo17) along with two teosintes (Zea diploperennis and Zea mays L. ssp. Parviglumis) were carried out at the NCSU phytotron. Plants for this report were grown in chambers at three day/night temperature regimes 22/18, 27/21 and 33/25 C under a 12h photoperiod. Leaf appearance parameters were recorded at 3-5 day intervals.

Plants of teosinte emerged with about 2-3 leaves whereas the maize plants had about 5 leaves at emergence. This may be associated with teosinte's smaller seed size and fewer cotyledonary leaves. The rate of leaf appearance among all genotypes was linear when plotted against accumulated temperature. Rates were generally similar for all genotypes (0.45 to 0.52 leaves per day at 33/25 C) indicating that there was little variation among the genotypes for vegetative development rates at near-optimum conditions. Moreover the vegetative developmental response to temperature appears conserved between maize and teosinte.

The number of leaves produced prior to flowering was largely independent of temperature and ranged from about 15 to 24. This indicated considerable genetic variation in the length of the vegetative phase among the races. Variation in final leaf numbers combined with similar leaf development rates among genotypes indicated that earliness was almost exclusively due to genetic variation for inherent earliness (time of floral initiation) rather than a differential response to temperature.

Propagation Of Photoperiod-Sensitive Tropical Maize Inbreds

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We have developed several maize recombinant inbred populations that are segregating for photoperiod sensitivity. The extremely photoperiod sensitive lines in our population are consistently barren when grown under field conditions in North Carolina because the long-days during the growing season set a non-optimal circadian rhythm in the plants. We used the phytotron to overcome the sterility block that occurs under non-optimal growing conditions by regulating day-length in a growth chamber. We successfully induced the photoperiod-sensitive lines to a short-day (11 hour photoperiod) circadian rhythm in a phytotron B growth chamber. These plants were later transferred to a greenhouse, where they grew to maturity and produced fertile pollen and silks. Because of the phytotron, we have successfully perpetuated the progeny of these extremely photoperiod sensitive lines, which will allow us to fine-map the genes underlying photoperiod sensitivity in maize.

Investigation Of Novel Mechanisms Of Cotton Fiber Development

C.H. Haigler Lab

The Phytotron continues to be essential to the progress of my research program at NCSU through providing the only controlled plant growth facilities to which I have access. Without these facilities, my research program would not be possible. I expect that 2007 will result in peer-reviewed publications describing the new research approaches initiated when I moved to NCSU in mid-2003. In 2006, only one abstract related to the use of the Phytotron was presented at the annual meeting of the American Society of Plant Biology and published:

> Singh B, Landgraf J, Wilkerson C, Haigler C. 2006. Genes and pathways associated with secondary wall deposition in cotton fibers. *Abstract Book: Plant Biology 2006*, p. 190, http://abstracts.aspb.org/pb2006/public/P18/P18018.html

This same poster was presented at the Gordon Conference on Plant Cell Walls, Univ. New England, Biddeford ME, July 30 – August 4.

However, the Phytotron was essential to the production of results that will provide the foundation for full manuscripts in 2007. Using the Arabidopsis growth room, we grew numerous T-DNA mutants in genes homologous to ones up-regulated for cotton fiber secondary wall deposition. Tissues were collected which are currently being analyzed for changes in cell wall deposition. During 2006, a new Ph.D. graduate student in Plant Biology, Lissette Betancur, became involved in this research. In a second use of this room, Utku Avci, Ph.D. student in Crop Science, grew Arabidopsis mutants that were analyzed for changes in xylem differentiation. This project was collaborative with Eric Beers, Virginia Polytechnical Institute. This work is the main focus of Utku's dissertation, which will be submitted in 2007. Full papers on two of his projects are currently in final stages of preparation.

Using the 26/22°C greenhouse, we grew cotton plants in order to collect ovules on the day of anthesis for establishing cotton tissue cultures. These experiments are part of our continuing work on an NCSU Invention Disclosure, #06-022, Novel Mechanisms Regulating Cotton Fiber Development. We manipulated the tissue cultures in various ways to add depth to the data proving the new mechanisms. We also collected bolls of various ages that had matured on the

plants in order to analyze morphological aspects of fiber development by light and electron microscopy. Ph.D. student, Utku Avci, and a Research Associate, Dr. Bir Singh, carried out this research. The tight temperature control in this greenhouse is essential to the reproducibility of our work on cotton fiber development. We also tried to grow ancestral/diploid cotton varieties in this greenhouse, but we found that the night interruption did not support their flowering. We appreciated the Phytotron staff allowing us to try a growth chamber in order to induce flowering, and are happy that we are observing flowering under long nights. The exotic cotton germplasm will be part of future grant proposals, and it is important to establish that we can grow them to boll maturity and to collect some preliminary data.

Evaluation Of *Nicotiana Tabacum* Genotypes Possessing *N. Africana*-Derived Genetic Tolerance To *Potato Virus Y*

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Abstract

New sources of genetic variability influencing resistance to *potato virus Y* (PVY) would be of value for developing tobacco (*Nicotiana tabacum* L.) cultivars resistant to this economically important pathogen. Materials possessing an introgressed genomic region (*Nafr*) from *N. africana* Merx. & Buttler were previously generated that exhibit tolerance to a severe isolate of PVY. The first objective of this research was to evaluate this source of variability for its effectiveness against an array of nine PVY isolates of different origin and severity. Seven near-isogenic genotypes of tobacco cultivar 'K326' were produced that possessed *Nafr* and the recessive potyvirus resistance gene, *va*, in different combinations and zygosities. In growth chamber experiments, *Nafr* provided little effect against mild non-necrotic isolates of PVY, but imparted significant increased resistance against the necrotic effects of two severe isolates, PVY N^SN^R and VAM-B. Data indicated that *Nafr* and *va* can be combined into single genotypes to increase the range and level of resistance/tolerance to PVY in tobacco.

Introduction

Potato virus Y (PVY) belongs to the largest plant virus family, *Potyviridae*, and is one of the most economically-important pathogens affecting tobacco (*Nicotiana tabacum* L.) production worldwide (Lucas, 1975). Genetic resistance offers the most efficient means of reducing economic loss from crop plant viruses. Extremely high levels of resistance to many viruses can be generated in a number crop species, including tobacco, using transgenic expression of virus-derived nucleotide sequences (Sudarsono et al., 1995). Current international objection to genetically-engineered tobacco cultivars makes commercialization of these technologies

difficult, however. Resistance to many PVY isolates (as well as other potyviruses such as *tobacco etch virus* and *tobacco vein mottling virus*) is conferred by irradiation-induced recessive alleles at the *va* locus in 'Virgin A Mutant' (Koelle, 1961), and also by naturally occurring recessive alleles at this locus found in several other cultivars (Wernsman, 1992). Partial resistance has also been identified in gametoclonal variant NC602 (Witherspoon et al., 1991). No source provides complete resistance to all strains or isolates of PVY, however. New sources of genetic variability influencing resistance or tolerance to this important pathogen are therefore continually being sought.

Lucas et al. (1980) found an accession of the African *Nicotiana* species, *N. africana* Merxmüller and Buttler, to be immune to three strains of PVY. Through interspecific hybridization and backcrossing, an alien chromosome segment carrying a gene(s) conferring tolerance to a severe isolate of PVY was transferred from this species to *N. tabacum* (Lewis, 2005). Materials possessing this region are considered tolerant to this isolate because they resist systemic necrosis that occurs in PVY-susceptible genotypes. A set of random amplified polymorphic DNA (RAPD) markers contained within the introgressed region were also identified (Lewis, 2005).

PVY is a highly variable pathogen, and numerous strains elicit a wide array of reactions across varying tobacco genotypes. The effectiveness of the introgressed *N. africana* genomic region against diverse PVY isolates has not yet been reported. The first objective of this research was to investigate the effectiveness of the alien chromosome segment against a set of nine PVY isolates collected from diverse tobacco-growing regions of the world, and that differ widely in terms of symptom type and severity on tobacco. Evaluations were based on symptom development in environmentally-controlled growth chamber experiments. The alien segment was evaluated in hemizygous and homozygous condition, and also in combination with the recessive gene, *va*, derived from tobacco breeding line 'Greenville 136.' Since modifying genes can influence observed reactions to virus infections (Johnson et al., 1982), these experiments were carried out using nearly-isogenic materials possessing the genetic mechanisms in different combinations in a 'K326' genetic background.

Materials and Methods

Plant Material

To allow comparison of different resistance/tolerance gene combinations in a nearly isogenic background, both *va* and the introgressed *N. africana*-derived genomic region (hereafter referred to as *Nafr*) were transferred to PVY-susceptible flue-cured tobacco cultivar 'K326' using backcrossing. K326 *va/va* was developed by transferring the recessive *va* allele contained in burley tobacco breeding line 'Greenville 136' to K326 using eight backcrosses, followed by self-pollination to establish a homozygous *va/va* isoline.

A 2*n*=48 plant homozygous for *Nafr* was previously developed by Lewis (2005). The alien segment was transferred to K326 using five backcrosses with selection for resistance against the necrotic effects of an isolate of PVY strain N^SN^R (designated as NC78) maintained by the tobacco breeding program at North Carolina State University (Gooding and Tolin, 1973). *Nafr* is transmitted through male gametes at a low frequency (Lewis, 2005). To achieve homozygosity for the region, an array of maternally-derived haploid plants was generated from a PVY N^SN^R-tolerant BC_5F_1 individual using the method of Burk et al. (1979a). Haploid plants possessing *Nafr* were identified based on the presence of two *N. africana* RAPD marker loci, UBC119.927 and OPZ20.342 (Lewis, 2005). Recombination between PVY N^SN^R tolerance and these markers has not been observed (Lewis, 2005). Selected individuals were chromosome doubled using the method of Kasperbauer and Collins (1972). PVY tolerance of resulting doubled haploid lines was verified via inoculation with PVY isolate NC78. One line, designated as DH04B-702-1, was selected to represent K326 *Nafr*/*Nafr* in this research (Table 1).

To combine *va* with *Nafr*, DH04B-702-1 was hybridized with K326 *va*/*va*. An array of maternally-derived haploid plants was generated from this cross, and individuals carrying *Nafr* were selected based on genotypes at the UBC119.927 and OPZ20.342 RAPD marker loci. These individuals were chromosome-doubled, and derived doubled haploid lines were inoculated with PVY N^SN^R (isolate NC78) to identify *Nafr*/*Nafr* lines that were also homozygous for *va*. Lines carrying only *Nafr* are resistant to the necrotic effects of this isolate, but exhibit obvious leaf mottling within 10 days after inoculation. *Nafr*/*Nafr va*/*va* genotypes exhibit an obviously superior level of resistance to the NC78 isolate of PVY N^SN^R, and typically exhibit no symptoms until about 16 days, when slight breakdown of the *va*/*va* mechanism occurs. Doubled haploid

line DH05B-515-15 was selected to represent the K326 *Nafr*/*Nafr va*/*va* genotype for this investigation (Table 1).

Various crosses between K326 and the three nearly isogenic lines described above were used to produce four F_1 hybrids carrying *va* and *Nafr* in different combinations and zygosities (Table 1). K326 was hybridized with K326 *va/va* to create an F_1 hybrid designated as K326 *Va/va*. DH04B-702-1 was crossed with K326 to produce an F_1 hybrid hemizygous for *Nafr* (K326 *Nafr*/---). DH05B-515-15 was hybridized with K326 *va*/*va* to produce a K326 *Nafr*/--- *va/va* F_1 hybrid. Finally, DH05B-515-15 was crossed with K326 to produce a K326 *Nafr*/--- *Va*/*va* F_1 hybrid.

Virus Isolates and Inoculations

Nine PVY isolates were selected to represent diversity in origin, symptom development on *N. tabacum* (ranging from mild mottling to systemic necrosis), and reaction on *va*/*va* genotypes (some *va*-breaking strains were selected). These isolates and their descriptions are listed in Table 2. Virus isolates were maintained in tobacco cultivar 'Burley 21' in insect-proof cages in a greenhouse, and inoculum was prepared by macerating systemically infected leaf in phosphate buffer (0.05 M Na_2HPO_4-KH_2PO_4, pH 7.2, 1 g tissue : 5 ml buffer) using a mortar and pestle. Approximately 1% (w/v) carborundum (600 mesh) was added to the inoculum, and inoculum was filtered through cheesecloth and maintained on ice prior to use. A mock inoculation treatment that consisted of only phosphate buffer and carborundum was also used. Plants were inoculated an average of 30 days after seeding (when plants were approximately 9 cm in diameter). Inoculum was applied to the entire adaxial surface of the two newest leaves per plant using cotton-tipped applicators.

Plants were evaluated 21 days post-inoculation for percent stem/veinal necrosis, percent inter-veinal necrosis, percent mottling, and percent leaf cupping/distortion. After evaluation for these symptoms, all plants were severed at the soil level. Fresh weights of the above-soil plant material were recorded to provide an indication of the degree of stunting caused by the PVY isolates.

Growth Chamber Experimental Design

The inoculation experiment was conducted in a 3 × 3 m walk-in controlled-environment growth chamber in the Southeastern Plant Environment Laboratory, Raleigh, NC. A split plot

experimental design with three replications was used. The main-plot factor consisted of the virus inoculation treatment (Table 2), and the sub-plot factor consisted of the plant genotype (Table 1). Inoculation treatments were randomized within replications, and genotypes were randomized within inoculation treatments. The experiment was repeated three times. Experimental units were single plants grown in 600 mL round pots filled with a soil mix comprised of 50% river bottom sand and 50% Redi-earth Plug and Seedling Mix (Sun Gro Horticulture, Bellevue, WA). The chamber was maintained at 25 +/- 0.5 °C with a 18 h light/6 h dark photoperiod. Plants were watered with a 0.5 × standard nutrient solution once or twice daily, depending on the stage of plant growth.

Statistical Analyses

For the growth chamber experiment, an analysis of variance (ANOVA) appropriate for a split-plot design was conducted using mixed model procedures facilitated by PROC MIXED of SAS (SAS Institute, Cary, NC). Main-plots and sub-plots were considered as fixed factors and repetitions were considered as random. The ANOVA was used to test the main effects and their interactions on the measured characteristics. Main-plot, sub-plot, and main-plot × sub-plot means were generated using the LSMEANS statement. Mean separations for main-plots and sub-plots were conducted using LSD tests at the 0.05 and 0.01 probability levels. Within each main-plot, all possible pairwise comparisons between genotypes were also made via *t*-tests generated by ESTIMATE statements in PROC MIXED.

Results

Growth Chamber Experiment

Symptoms on inoculated plants in the growth chamber experiment were diverse, ranging from mild leaf mottling with little stunting to very severe systemic necrosis and plant death. Significant differences were observed between inoculation treatments and genotypes for all of the measured characters. Isolates having the greatest impact on overall plant growth (as reflected by reduced fresh weight) were those that induced severe necrotic effects, such as N^SN^R, M^SN^R, and VAM-B (Table 4). The isolates PVY M^SM^R, 99-04, Y^NW PL1, and Y^NW PL2 produced

leaf mottling with little to no necrotic effects, and did not significantly reduce fresh weights relative to the mock inoculation treatment (Table 3).

Averaged over all six inoculation treatments, six genotypes exhibited significantly greater fresh weight than K326 (Table 4). Genotype K326 *Nafr*/*Nafr va*/*va* produced the greatest fresh weight, although this was not significantly greater than the fresh weights for K326 *va*/*va*, K326 *Nafr*/--- *va*/*va*, or K326 *Nafr*/--- *Va*/*va*. K326 displayed significantly higher levels of stem/veinal and inter-veinal necrosis relative to all other genotypes. Genotypes K326 *Nafr*/*Nafr va*/*va* and K326 *Nafr*/--- *va*/*va* exhibited levels of leaf mottling that were significantly lower than that for all other genotypes (Table 4). K326 *Nafr*/*Nafr* exhibited higher levels of resistance to necrotic effects, leaf mottling, and stunting relative to K326 *Nafr*/---. Most of these differences were not statistically significant, however.

For many PVY isolates, symptom development was highly dependent upon plant genotype. Comparisons of greatest interest are therefore those within specific inoculation treatments. All possible pairwise comparisons were made between genotypes within individual inoculation treatments using simple *t*-tests. Results from within-inoculation treatments are discussed below in the general order of increasing isolate severity.

PVY Y^NW PL1 and Y^NW PL2

PVY isolates Y^NW PL1 and Y^NW PL2 produced very similar symptoms in the growth chamber experiment and induced slight stem necrosis, mild leaf mottling, and slight leaf cupping on K326 and *Va*/*va* genotypes (Table 5). Significant differences between genotypes were only observed for leaf mottling, leaf cupping/distortion, and fresh weight. For both Y^NW PL1 and Y^NW PL2, *va*/*va* genotypes exhibited significantly lower levels of leaf mottling relative to K326 and K326 *Nafr*/*Nafr*. For isolate PVY Y^NW PL2, *va*/*va* genotypes exhibited significantly lower levels of leaf cupping/distortion relative to K326, K326 *Nafr*/*Nafr*, and K326 *Nafr*/---. Interestingly, for both Y^NW PL1 and Y^NW PL2, *Va*/*va* heterozygotes were not significantly different from K326 *va*/*va* for leaf mottling (Table 6). For Y^NW PL2, K326 *Va*/*va* exhibited significantly less mottling relative to K326. Also for Y^NW PL2, the fresh weights for K326 *Nafr*/*Nafr* and K326 *Nafr*/--- *va*/*va* were significantly lower than those for K326 *Va*/*va* and K326 *va*/*va*. Neither K326 *Nafr*/*Nafr* nor K326 *Nafr*/--- was significantly different from K326 for any of the evaluated characteristics

PVY $M^S M^R$

PVY isolate $M^S M^R$ did not induce necrotic effects on any genotype, but produced moderate levels of leaf mottling across all genotypes (Table 5). The degree of leaf mottling displayed by K326 *va*/*va* and K326 *Nafr*/--- *va*/*va* was significantly lower relative to all other genotypes. Neither K326 *Nafr*/*Nafr* nor K326 *Nafr*/--- were significantly different from K326 for any of the measured symptoms.

PVY 99-04

Slight stem necrosis and mild leaf mottling was observed for all genotypes inoculated with PVY isolate 99-04 (Table 5). No significant differences were found among genotypes for any of the five characteristics that were evaluated, however. Therefore, neither *va*, *Nafr*, or any combination of these genetic factors provided any statistically significant resistance/tolerance to this isolate.

PVY DL

PVY isolate DL produced moderate leaf mottling and leaf cupping/distortion on all genotypes, with little to no stem/veinal or inter-veinal necrosis (Table 5). No significant differences were found between any of the genotypes for any of the evaluated symptoms.

PVY Europe H

PVY isolate Europe H produced moderate stem/veinal necrosis and leaf mottling on K326 and *Va*/*va* genotypes (Table 5). All *va*/*va* genotypes exhibited significantly less stem/veinal necrosis and leaf mottling compared to K326 and *Va*/*va* genotypes. K326 *va*/*va* *Nafr*/*Nafr* produced the highest fresh weight (significantly greater than fresh weights for K326, K326 *Nafr*/*Nafr*, K326 *Nafr*/---, and K326 *Nafr*/--- *Va*/*va*). Neither K326 *Nafr*/*Nafr* nor K326 *Nafr*/--- were significantly different from K326 for any of the measured symptoms.

PVY VAM-B

PVY isolate VAM-B produced slight necrotic effects in the stem and vascular tissue of K326, K326 *va*/*va*, and K326 *Va*/*va*. None was observed in any genotype containing *Nafr*, however (Table 5). Medium levels of inter-veinal necrosis, leaf mottling, and stunting (as

reflected by lower fresh weights) were also exhibited in K326, K326 *va*/*va* and K326 *Va*/*va*. Significantly lower levels were observed in *Nafr*-containing genotypes. No significant differences were observed between K326 *Nafr*/*Nafr* and K326 *Nafr*/--- for any characteristic.

PVY N^SN^R

PVY N^SN^R produced extremely severe necrotic effects on K326 and K326 *Va*/*va*. Genotypes possessing *Nafr* exhibited few necrotic effects in vascular tissues and were significantly resistant to these effects in comparisons with K326 and K326 *Va*/*va* (Table 5). K326 *va*/*va* exhibited greater resistance to these necrotic effects as compared to K326, but was not as resistant as materials carrying *Nafr*. K326 *va*/*va Nafr*/*Nafr* displayed significantly less leaf cupping/distortion relative to K326 *Nafr*/*Nafr*. All genotypes produced significantly greater fresh weight relative to K326, K326 *Va*/*va*, and K326 *Nafr*/---. K326 *Nafr*/*Nafr* was not significantly better than K326 *Nafr*/--- for stem/veinal necrosis, but did exhibit significantly greater fresh weight and a significantly lower level of inter-veinal necrosis.

PVY M^SN^R

All genotypes were affected by severe systemic necrosis after inoculation with PVY isolate M^SN^R (Table 5). K326 and K326 *Nafr*/--- exhibited significantly greater levels of stem/veinal and inter-veinal necrosis compared to all other genotypes. Genotype K326 *Nafr*/--- *va*/*va* exhibited significantly lower levels of stem/veinal necrosis relative to all other entries. K326 *Nafr*/*Nafr va*/*va* exhibited significantly lower levels of inter-veinal necrosis relative to K326 *va/va*. In pairwise comparisons, fresh weights of *va*/*va* genotypes were significantly greater than fresh weights for K326 or *Va*/*va* genotypes.

Discussion

Symptoms observed on plants inoculated with the nine PVY isolates were highly variable and often genotype-dependent. No genotype exhibited complete resistance to any isolate. Relative to *Nafr*, the recessive *va* resistance mechanism had a tendency to provide greater resistance or tolerance against leaf mottling and leaf distortion caused by the more mild PVY isolates such as M^SM^R, Y^NW PL1, and Y^NW PL2. Relative to K326, *va*/*va* genotypes exhibited

increased resistance against the necrotic effects of isolates Europe H, $Y^{N}W$ PL1, $Y^{N}W$ PL2, and PVY $N^{S}N^{R}$. The *va* mechanism had little effect against necrosis induced by PVY isolate VAM-B, however. The introgressed *Nafr* region had little effect on leaf mottling caused by the mild isolates, but had very significant effects against the necrotic effects caused by severe isolates $N^{S}N^{R}$ and VAM-B. *Nafr*/*Nafr* genotypes exhibited much greater resistance to the necrotic effects caused by these two isolates as compared to K326 *va*/*va*. Neither *Nafr* nor *va* was able to alleviate the severe systemic necrosis induced by PVY isolate $M^{S}N^{R}$, however. K326 is homozygous for the dominant gene, *Rk*, which provides resistance against the root knot nematode, *Meloidogyne incognita*. Development of systemic necrosis in tobacco genotypes carrying *Rk* is typical upon inoculation with PVY $M^{S}N^{R}$ (Burk et al., 1979b). Rufty et al. (1983) proposed that the necrotic response in *Rk* genotypes is due to pleiotropic effects of this resistance gene.

Breeding lines possessing *Nafr* may provide an additional source of genetic variability for developing tobacco cultivars with improved resistance to PVY. The results of this investigation demonstrate that *va* and *Nafr* can be combined into single genotypes to increase the range and level of resistance/tolerance to this economically important pathogen. Although slight resistance might be gained against certain effects caused by some PVY isolates in *Va*/*va* genotypes, it is generally assumed that homozygous recessive genotypes are necessary to obtain the greatest level of resistance from this mechanism. In the work described here, small differences were found between *Nafr*/*Nafr* and *Nafr*/--- genotypes for resistance to PVY VAM-B. For PVY $N^{S}N^{R}$, however, *Nafr*/*Nafr* genotypes displayed lower levels of stunting and inter-veinal necrosis relative to *Nafr*/--- hemizygotes.

Finally, it is important to note that the full level of resistance to PVY that has been reported for *N. africana* (Lucas et al., 1980) was not observed in the lines described here. It is not known if the full resistance expressed by this species is controlled by multiple genes on more than one chromosome of the *N. africana* genome, or if the resistance gene introgressed in our materials is influenced in a negative manner by modifying genes contained within the *N. tabacum* genome. Experiments are underway to better understand the genetics controlling the very high level of resistance exhibited by *N. africana*.

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Table 1. Genotypes used in experiments to evaluate K326 and derived isolines for reactions to PVY inoculation.

Genotype	Pedigree
K326	Certified Seed
K326 *va*/*va*	K326 *va*/*va* BC_8S_3
K326 *Nafr*/*Nafr*	DH04B-702-1 (doubled haploid line derived from the BC_5F_1 generation of backcrossing)
K326 *Nafr*/*Nafr va*/*va*	DH05B-515-15 (doubled haploid line derived from the BC_6F_1 generation of backcrossing)
K326 *Va*/*va*	K326 *va*/*va* BC_7S_3 × K326; F_1 Hybrid
K326 *Nafr*/---	DH04B-702-1 × K326; F_1 Hybrid
K326 *Nafr*/--- *va*/*va*	DH05B-515-15 × K326 *va*/*va* F_1 Hybrid
K326 *Nafr*/--- *Va*/*va*	DH05B-515-15 × K326; F_1 Hybrid

Table 2. PVY inoculation treatments used in research to investigate genetic resistance/tolerance in K326 and derived isolines.

Isolate	Country of Origin	Donor	Comments
PVY M^SM^R	U.S.A.	North Carolina State University	Causes mild symptoms on root knot nematode-susceptible and -resistant genotypes (Gooding and Tolin, 1973).
PVY N^SN^R (NC78)	U.S.A.	North Carolina State University	Causes necrotic symptoms on root knot nematode-susceptible and -resistant genotypes (Gooding and Tolin, 1973).
PVY M^SN^R (NC138)	U.S.A	North Carolina State University	Causes mild symptoms on root knot nematode-susceptible genotypes, but necrotic symptoms on root-knot nematode-resistant genotypes (Gooding and Tolin, 1973).
PVY VAM-B (NC182)	U.S.A.	North Carolina State University	Necrotic isolate that breaks resistance of Virginia A Mutant (Gooding, 1985).
PVY DL	U.S.A.	North Carolina State University	Isolate that breaks *va*-mediated resistance in burley tobacco cultivar TN86 (Pesic-Van Esbroeck et al., 1997).
PVY Europe H (NC189)	Hungary	North Carolina State University	Type isolate of necrotizing strain from tobacco in Hungary (Gooding, 1985).
PVY 99-04	France	Jean-Louis Verrier, Altadis, Bergerac, France	Necrotic isolate that does not overcome *va* in France (Jean Louis Verrier, personal communication).
PVY Y^NW PL1	Poland	Teresa Doroszewska, Pulawy Institute for Soil Science and Plant Cultivation, Poland	Recombinant PVY isolate collected from tobacco in Poland (Teresa Doroszewska, personal communication).
PVY Y^NW PL2	Poland	Teresa Doroszewska, Pulawy Institute for Soil Science and Plant Cultivation, Poland	Recombinant PVY isolate collected from tobacco in Poland (Teresa Doroszewska, personal communication).
Mock Inoculation	-------	-------	Comprised of inoculation buffer plus carborundum.

Table 3. Main-plot (inoculation treatment) means (averaged over all genotypes).

Inoculation Treatment	% stem/veinal necrosis	% inter-veinal necrosis	% leaf mottling	% leaf cupping/ distortion	fresh weight (g)
PVY M^SM^R	0.0	0.0	22.0	21.1	40.7
PVY N^SN^R (NC78)	21.3	27.7	16.8	19.6	26.3
PVY M^SN^R (NC138)	56.1	47.6	20.3	32.9	12.2
PVY VAM-B (NC182)	1.7	12.2	30.1	15.9	31.1
PVY DL	1.2	0.0	21.5	12.5	36.3
PVY Europe H (NC189)	14.8	1.7	8.2	6.8	33.9
PVY 99-04	4.0	0.0	7.4	1.8	39.6
PVY Y^NW PL1	2.1	0.0	4.7	1.0	41.3
PVY Y^NW PL2	2.1	0.0	6.3	1.9	40.1
Mock Inoculation	0.0	0.0	0.0	0.0	41.6
LSD (0.01), among main-plot means	5.5	3.9	6.5	6.6	3.5
LSD (0.05), among main-plot means	4.1	2.9	4.9	5.0	2.6

Table 4. Sub-plot (genotype) means (averaged over inoculation treatments).

Genotype	% stem/veinal necrosis	% inter-veinal necrosis	% leaf mottling	% leaf cupping/ distortion	fresh weight (g)
K326	21.2	19.3	16.2	15.6	29.6
K326 *va*/*va*	6.7	7.8	12.6	10.0	37.3
K326 *Nafr*/*Nafr*	9.8	5.3	14.4	12.6	33.1
K326 *Nafr*/*Nafr va*/*va*	4.9	2.4	9.9	8.3	37.6
K326 *Va*/*va*	16.7	17.2	15.5	11.8	31.6
K326 *Nafr*/---	11.3	10.3	15.0	13.2	32.2
K326 *Nafr*/--- *va*/*va*	3.5	2.6	10.1	7.3	37.1
K326 *Nafr*/--- *Va*/*va*	8.6	6.6	16.0	11.6	35.9
LSD (0.01), among sub-plot means	3.4	3.0	2.8	3.2	2.6
LSD (0.05), among sub-plot means	2.6	2.3	2.1	2.4	2.0

Table 5. Inoculation treatment (main-plot) × genotype (sub-plot) means.

Inoculation Treatment	Genotype	% stem/veinal necrosis	% inter-veinal necrosis	% leaf mottling	% leaf cupping/ distortion	fresh weight (g)
PVY M^SM^R	K326	0.0	0.0	25.0	27.8	39.0
PVY M^SM^R	K326 *va*/*va*	0.0	0.0	11.4	5.6	46.4
PVY M^SM^R	K326 *Nafr*/*Nafr*	0.0	0.0	26.7	28.3	39.0
PVY M^SM^R	K326 *Nafr*/*Nafr va*/*va*	0.0	0.0	22.2	17.8	39.8
PVY M^SM^R	K326 *Va*/*va*	0.0	0.0	26.1	21.1	41.7
PVY M^SM^R	K326 *Nafr*/---	0.0	0.0	26.7	29.8	37.4
PVY M^SM^R	K326 *Nafr*/--- *va*/*va*	0.0	0.0	14.4	12.8	41.6
PVY M^SM^R	K326 *Nafr*/--- *Va*/*va*	0.0	0.0	23.3	25.6	41.0
PVY N^SN^R (NC78)	K326	83.1	83.7	7.8	38.9	4.9
PVY N^SN^R (NC78)	K326 *va*/*va*	10.0	16.4	12.2	21.1	34.2
PVY N^SN^R (NC78)	K326 *Nafr*/*Nafr*	0.3	0.6	17.8	17.2	36.0
PVY N^SN^R (NC78)	K326 *Nafr*/*Nafr va*/*va*	0.0	0.0	7.8	0.9	37.4
PVY N^SN^R (NC78)	K326 *Va*/*va*	75.0	79.4	10.0	30.0	4.6
PVY N^SN^R (NC78)	K326 *Nafr*/---	0.6	22.0	33.9	25.6	22.6
PVY N^SN^R (NC78)	K326 *Nafr*/--- *va*/*va*	0.0	0.0	12.6	6.1	37.7
PVY N^SN^R (NC78)	K326 *Nafr*/--- *Va*/*va*	1.7	19.4	32.2	17.2	32.7
PVY M^SN^R (NC138)	K326	78.1	73.9	13.3	31.1	5.6
PVY M^SN^R (NC138)	K326 *va*/*va*	46.1	31.7	20.0	38.3	20.7
PVY M^SN^R (NC138)	K326 *Nafr*/*Nafr*	58.9	50.6	23.9	37.8	7.7
PVY M^SN^R (NC138)	K326 *Nafr*/*Nafr va*/*va*	41.1	22.8	22.2	36.7	22.4
PVY M^SN^R (NC138)	K326 *Va*/*va*	58.3	57.8	20.6	22.2	7.6
PVY M^SN^R (NC138)	K326 *Nafr*/---	80.3	76.1	12.8	32.8	2.7
PVY M^SN^R (NC138)	K326 *Nafr*/--- *va*/*va*	31.7	25.6	20.9	26.7	22.3
PVY M^SN^R (NC138)	K326 *Nafr*/--- *Va*/*va*	53.9	42.2	28.3	37.8	8.7
PVY VAM-B (NC182)	K326	5.6	31.4	46.1	22.8	19.7
PVY VAM-B (NC182)	K326 *va*/*va*	3.3	30.0	51.1	23.3	18.4
PVY VAM-B (NC182)	K326 *Nafr*/*Nafr*	0.0	0.0	15.6	9.4	39.6
PVY VAM-B (NC182)	K326 *Nafr*/*Nafr va*/*va*	0.0	1.1	20.0	11.7	35.6
PVY VAM-B (NC182)	K326 *Va*/*va*	5.0	31.7	48.3	27.2	14.4
PVY VAM-B (NC182)	K326 *Nafr*/---	0.0	2.8	16.7	9.4	37.4
PVY VAM-B (NC182)	K326 *Nafr*/--- *va*/*va*	0.0	0.0	22.2	12.2	37.4
PVY VAM-B (NC182)	K326 *Nafr*/--- *Va*/*va*	0.0	0.9	20.6	11.1	45.9
PVY Europe H (NC189)	K326	27.8	3.9	15.1	12.3	27.8
PVY Europe H (NC189)	K326 *va*/*va*	5.6	0.0	2.8	1.1	38.9
PVY Europe H (NC189)	K326 *Nafr*/*Nafr*	23.9	1.7	12.3	12.2	28.2
PVY Europe H (NC189)	K326 *Nafr*/*Nafr va*/*va*	4.4	0.0	2.2	1.4	43.6
PVY Europe H (NC189)	K326 *Va*/*va*	17.8	2.8	10.0	6.8	32.5
PVY Europe H (NC189)	K326 *Nafr*/---	18.9	1.7	12.0	11.3	30.0
PVY Europe H (NC189)	K326 *Nafr*/--- *va*/*va*	0.9	0.0	2.6	1.1	38.6
PVY Europe H (NC189)	K326 *Nafr*/--- *Va*/*va*	18.9	3.9	8.9	7.8	31.9
PVY 99-04	K326	7.2	0.0	10.6	3.4	36.5

PVY 99-04	K326 *va*/*va*	1.4	0.0	5.4	0.0	44.1
PVY 99-04	K326 *Nafr*/*Nafr*	5.4	0.0	9.7	2.0	35.8
PVY 99-04	K326 *Nafr*/*Nafr va*/*va*	1.8	0.0	3.7	0.6	42.2
PVY 99-04	K326 *Va*/*va*	4.7	0.0	7.0	0.0	42.3
PVY 99-04	K326 *Nafr*/---	5.1	0.0	11.1	3.4	37.2
PVY 99-04	K326 *Nafr*/--- *va*/*va*	1.4	0.0	4.1	0.6	41.0
PVY 99-04	K326 *Nafr*/--- *Va*/*va*	5.2	0.0	7.3	0.9	37.6
PVY Y^{N}W PL1	K326	3.9	0.0	7.9	2.6	41.3
PVY Y^{N}W PL1	K326 *va*/*va*	0.0	0.0	0.3	0.0	41.9
PVY Y^{N}W PL1	K326 *Nafr*/*Nafr*	4.4	0.0	8.1	2.4	35.9
PVY Y^{N}W PL1	K326 *Nafr*/*Nafr va*/*va*	0.3	0.0	0.6	0.0	43.1
PVY Y^{N}W PL1	K326 *Va*/*va*	2.2	0.0	7.0	0.3	44.0
PVY Y^{N}W PL1	K326 *Nafr*/---	3.8	0.0	7.9	2.1	41.0
PVY Y^{N}W PL1	K326 *Nafr*/--- *va*/*va*	0.3	0.0	0.6	0.0	43.1
PVY Y^{N}W PL1	K326 *Nafr*/--- *Va*/*va*	1.9	0.0	5.4	0.3	42.5
PVY Y^{N}W PL2	K326	4.3	0.0	13.1	4.6	39.8
PVY Y^{N}W PL2	K326 *va*/*va*	0.0	0.0	0.6	0.3	46.2
PVY Y^{N}W PL2	K326 *Nafr*/*Nafr*	3.9	0.0	11.1	3.8	35.0
PVY Y^{N}W PL2	K326 *Nafr*/*Nafr va*/*va*	0.9	0.0	0.8	0.0	40.7
PVY Y^{N}W PL2	K326 *Va*/*va*	2.0	0.0	5.4	0.3	45.3
PVY Y^{N}W PL2	K326 *Nafr*/---	3.0	0.0	9.2	4.3	37.9
PVY Y^{N}W PL2	K326 *Nafr*/--- *va*/*va*	0.0	0.0	0.8	0.3	36.4
PVY Y^{N}W PL2	K326 *Nafr*/--- *Va*/*va*	2.7	0.0	9.0	1.4	39.3
PVY DL	K326	1.9	0.3	23.3	12.8	39.1
PVY DL	K326 *va*/*va*	1.0	0.0	21.7	10.0	38.6
PVY DL	K326 *Nafr*/*Nafr*	0.8	0.0	18.9	12.8	33.1
PVY DL	K326 *Nafr*/*Nafr va*/*va*	1.1	0.0	20.6	13.7	33.5
PVY DL	K326 *Va*/*va*	1.6	0.0	20.6	10.0	38.9
PVY DL	K326 *Nafr*/---	1.0	0.0	19.4	13.1	35.5
PVY DL	K326 *Nafr*/--- *va*/*va*	0.4	0.0	22.8	13.1	31.9
PVY DL	K326 *Nafr*/--- *Va*/*va*	1.6	0.0	24.4	14.2	39.6
Mock Inoculation	K326	0.0	0.0	0.0	0.0	42.2
Mock Inoculation	K326 *va*/*va*	0.0	0.0	0.0	0.0	43.6
Mock Inoculation	K326 *Nafr*/*Nafr*	0.0	0.0	0.0	0.0	40.8
Mock Inoculation	K326 *Nafr*/*Nafr va*/*va*	0.0	0.0	0.0	0.0	40.5
Mock Inoculation	K326 *Va*/*va*	0.0	0.0	0.0	0.0	44.8
Mock Inoculation	K326 *Nafr*/---	0.0	0.0	0.0	0.0	40.2
Mock Inoculation	K326 *Nafr*/--- *va*/*va*	0.0	0.0	0.0	0.0	41.1
Mock Inoculation	K326 *Nafr*/--- *Va*/*va*	0.0	0.0	0.0	0.0	39.9
LSD (0.01), for comparison of two genotype means within the same inoculation treatment		10.8	9.5	8.8	10.2	8.3
LSD (0.05), for comparison of two genotype means within the same inoculation treatment		8.2	7.2	6.7	7.7	6.3
LSD (0.01), for comparison of two inoculation treatments for the same genotype		8.6	8.0	5.4	7.1	7.0
LSD (0.05), for comparison of two inoculation treatments for the same genotype		6.5	6.1	4.1	5.4	5.3

Transformation Of Grasses

Ron Qu
Crop Science

1. Two graduate students of mine, Shujie Dong and Jianli Lu, obtained their Ph.D. degree in 2006. Both of their projects involved heavily use of the NCSU Phytotron facilities. Their dissertations are published on line, which included the acknowledgments for the assistance of the Phytotron:

   http://www.lib.ncsu.edu/theses/available/etd-04242006-095937/
   http://www.lib.ncsu.edu/theses/available/etd-04272006-145216/unrestricted/etd.pdf

2. In Dr. Dong's research, she used the greenhouse and growth chambers in the Phytotron to evaluate transgenic tall fescue plants for resistance to fungal diseases or drought tolerance.

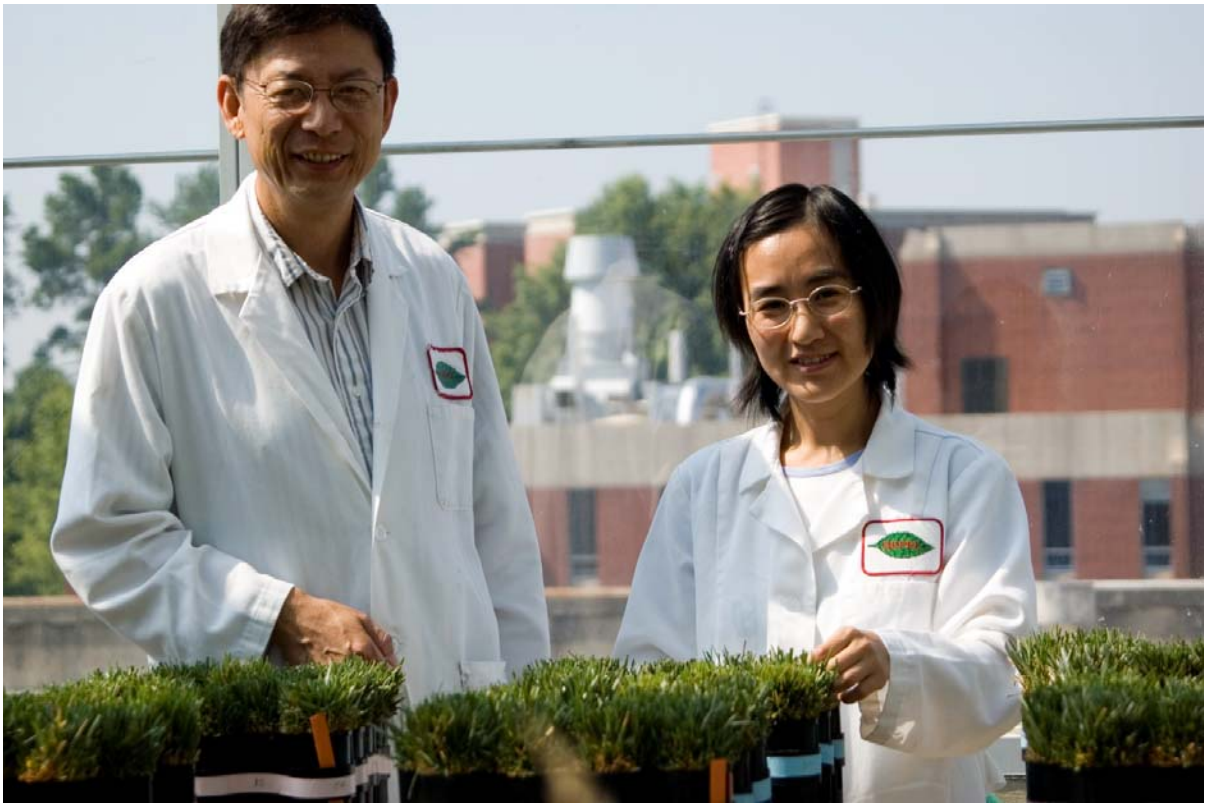
3. In 2006, the Phytotron provided space for Dr. Lu and for Tan Tuong to grow transgenic rice plants to evaluate the expression strength and pattern of a rice gene promoter we isolated. In that project, we also evaluated the effect of an intron on the promoter expression level and pattern. The uniformity of the Phytotron environment allowed us to obtain reliable data. Dr. Lu finished his experiments in 2006 and Tan is continuing his project by evaluating inheritance of transgene expression.

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

5. We grew wheat plants to provide experimental materials for a wheat transformation project aiming to improve its drought tolerance.

6. In 2006, the Phytotron also kindly provided space to my graduate student, Ruyu Li, to conduct her breeding project to improve cold tolerance of St. Augustinegrass.

The well-managed NCSU Phytotron is an extremely useful facility for the experiments in my lab. We appreciate very much the strong support and kind assistance we received in the past year from the Phytotron staff and the facility.



Effect Of Queen Mandibular Pheromone Exposure On Nestmate Recognition In Honey Bees.

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Social insects such as honey bees (*Apis mellifera*), use olfactory cues to distinguish between nestmates and conspecific nonnestmate intruders. These olfactory cues typically consist of cuticular hydrocarbons, and the composition of these cuticular hydrocarbon profiles are affected by genotype and environment (reviewed in Howard and Blomquist 2005). Individuals in the colony will attack non-nestmate individuals that do not possess the appropriate cuticular hydrocarbon profile; interestingly, removal of the hydrocarbons results in acceptance of the intruder individuals (Breed et al. 1998).

We determined whether nestmate responses to intruders were altered by exposure to queen mandibular pheromone (QMP). QMP is produced by the queen, and causes profound changes in worker behavior and physiology (Slessor et al. 2005). Previous studies demonstrated that increasing levels of octopamine improved the ability of bees to distinguish between nestmates and nonnestmates (Robinson et al. 1999); octopamine has also been shown to improve olfactory discrimination in learning and memory assays (Farooqui et al. 2004). Bees reared in the presence of a queen have improved performance in olfactory learning and memory assays compared to bees reared in queenless colonies (Morgan et al. 1998), suggesting that QMP may also improve olfactory discrimination and nestmate recognition. However, colony-level studies

demonstrated that there was no significant variation in the number of defensive stings by queenless colony workers exposed to synthetic QMP versus control queenless colonies, but there was a decrease in the number of bees recruited to the entrance of the colony by an intruder stimulus (Gervan et al. 2005), suggesting that QMP may generally act to reduce aggressiveness. In contrast, in fire ants, colonies with a live queen are more aggressive toward intruders than queenless colonies (Vander Meer and Alonso, 2002). Thus, QMP could modulate responses to intruder bees on a variety of levels: it could improve discrimination between nestmates and nonnestmates, it could reduce overall levels of aggression, or it could increase levels of aggression. In this study, we assayed the effect of QMP exposure on worker nestmate recognition.

Methods. Honey bee colonies were maintained according to standard commercial beekeeping procedures at the NCSU Lake Wheeler Honey Bee Research Facility. Colonies headed by single-drone inseminated queens (*Apis mellifera carnica*, Glenn Apiaries, CA) served as the primary sources of bees in these experiments. Colonies headed by naturally-mated Buckfast-SMR queens (obtained from B. Weaver Apiaries, TX) served as the source for the non-nestmate intruder bees. This ensured that the bees were from different genetic backgrounds, and thus should have very different cuticular hydrocarbon profiles. Brood frames from the two different source colonies were collected, and brood were allowed to emerge for 24 hours in a temperature and humidity controlled incubator (33˚C,~50% relative humidity). Newly emerged workers were placed in modified 10 cm Petri dishes, 10 worker bees/dish. 28 dishes were set up using bees from one colony, and an additional two cages each were set up with bees from either the same colony or a second colony, these bees served as the "intruder" nestmate and nonnestmate bees respectively. Dishes were maintained for seven days in a temperature and humidity controlled environmental chamber (33˚C ,~50% R.H., Phytotron, NCSU) under red light. Bees were fed 50% sucrose-water solution, provided fresh daily. Half of the test cages were treated with 0.1 queen equivalents of QMP (Pherotech, Canada). QMP was diluted in an isopropanol/1% water solution at 0.01 queen equivalents/uL. 10 uL of the diluted QMP was placed on a coverslip and the solvent was allowed to evaporate before being placed in the cages. This quantity of QMP is sufficient to elicit similar behavioral and physiological responses as a live queen (Grozinger et al. 2003, Hoover et al. 2002). QMP was applied at the same time each

day. The untreated control cages were exposed to a solvent control, as were the dishes of nestmate and nonnestmate intruder bees. This experiment was repeated 4 times.

On the seventh day of the experiment, bees were treated with QMP, and one hour later the nestmate recognition assay was performed, according to standard procedures (Breed 1983). A single intruder bee (either a nestmate or nonnestmate) was introduced to each cage. The percentage of cages with aggressive interactions (biting, stinging attempts) in the first four minutes after introduction were recorded. On the second day, the percentage of cages with a dead intruder bee was recorded. For cuticular hydrocarbon analyses, 10 workers from each group (with and without QMP) were submerged individually in 1ml pentane for 10 minutes with gentle agitation. Hydrocarbon compound identification was achieved by GC/MS. The initial oven temperature started at 150°C for 2 minutes, increased to 250°C at 15°C/min, and then increased to 300°C at 5°C/min, and was held there for 20 min. Quantification and chemical profile characterizations were done by GC peak integration using the relative abundance of the various peaks.

Results and Discussion. Our preliminary result showed that QMP did not change the aggression rate toward both nestmates and conspecific non-nestmates during the initial four minutes of the assay (Fig. 1A). However, nonnestmates introduced to QMP-treated cages were more likely to be killed than those introduced to untreated cages by the second day (Fig. 1B). We measured protein levels in the venom glands of untreated and QMP-treated bees to determine if QMP-treated bees simply had more venom; we did not find any significant differences in protein quantities (data not shown). We also measured the cuticular hydrocarbon profiles of the untreated and QMP-treated nestmates compared to the nonnestmate intruder bees, since one possible explanation for the increased aggression could be that the hydrocarbon profiles are strongly affected by QMP exposure. We found that the hydrocarbon profiles were significantly different between untreated and QMP-treated nestmates, but the difference was relatively small compared to the differences between bees of different genotypes (Fig 2).

Our results suggest that exposure of bees to QMP results in improved discrimination of nestmates and nonnestmates, and this improvement could be due to changes in the olfactory

discrimination abilities or the aggression of the treated workers, rather than due to large-scale changes in hydrocarbon profiles. We did not observe an obvious change in aggressive interactions in the initial stages of the assay, suggesting that QMP-treated bees are not necessarily more aggressive on average, but perhaps have higher relative levels of aggression (ie, more stinging vs. biting) or are more persistently aggressive. Additional studies need to be conducted to determine if QMP affects the level of aggression or the duration or aggression.

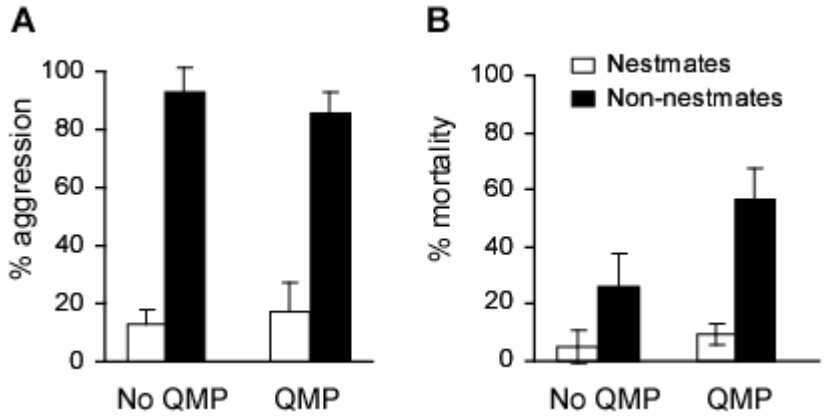

Figure 1. Effect of QMP on honey bee nestmate recognition. QMP-treated and untreated bees were equally aggressive towards nestmate and nonnestmates during the initial 4 min assay (A). However, non-nestmates introduced to QMP-treated cages were much more likely to be killed than those introduced to untreated cages (B). Bar indicates mean ± standard error (N=4 trials, 7 cages/treatment group/trial).

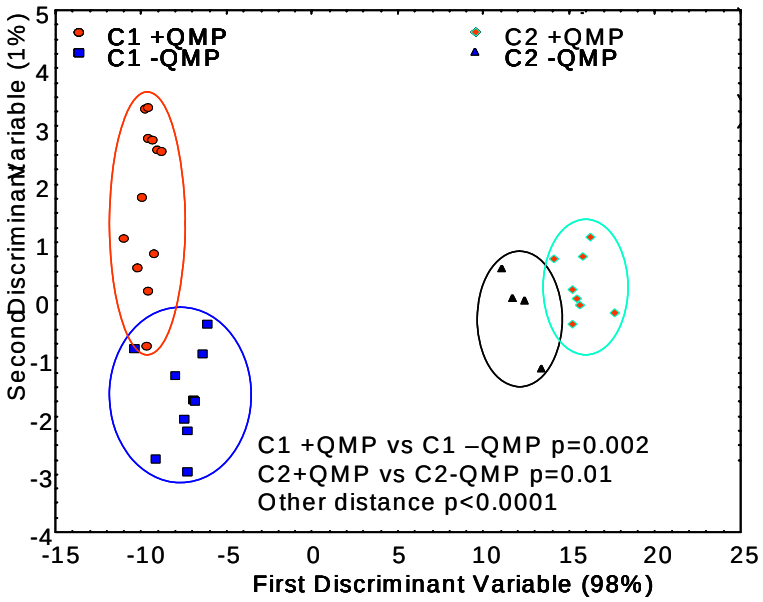

Figure 2. Principal components analysis of cuticular hydrocarbon profiles from QMP treated vs. untreated nestmate worker bees from two different colonies. We found that the hydrocarbon profiles were significantly different between QMP-treated (QMP+) or untreated (QMP-) bees (Colony 1: p=0.002, Colony 2: p=0.01). However, the difference was greatest between bees from two different colonies (C1 vs C2, p<0.0001).

Acknowledgements.

The behavioral assays were carried out in the Phytotron facilities at NCSU. We would like to thank Joe Flowers for expert beekeeping assistance, and Professors Coby Schal and Michael Roe for access to GC/MS instrumentation. F.J. Richard is supported by a post-doctoral fellowship from the W.M. Keck Center for Behavioral Biology. This work was funded by a grant from the NIH-NIDCD Gene Robinson (subaward to CMG, 1 R01 DC006395).

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Influence of Light and Temperature on Germination of Seeds of Pinkshell Azalea (*Rhododendron vaseyi*) from Two Locations in Western North Carolina

Anthony V. LeBude, Frank A. Blazich, Lela C. Walker, Joseph L. Conner, and Sonya M. Robinson

Introduction

The deciduous pinkshell azalea (*Rhododendron vaseyi* A. Gray) is a rare, ericaceous species endemic to only five counties in western North Carolina (3). May through June, plants produce attractive, pink to rarely white flowers in clusters of 3 to 15 flowers. Because natural habitats are diminishing for this plant due to home and road construction, protocols for seed germination are being investigated as a means to propagate the plant sexually.

In Fall 2005 a study was conducted in the Phytotron to investigate the influence of light and temperature on seed germination of *R. vaseyi* (6). Seeds of the species from one location in western North Carolina were germinated with various combinations of photoperiod and temperature treatments and the best treatment resulted in 50% germination (6). The low germination raised a number of questions, one being whether seed germination varies between populations/localities of the species. Therefore, the following research was conducted to study the influence of light and temperature on germination of seeds of *R. vaseyi* collected from two locations in western North Carolina.

Materials and Methods

On October 15, 2006, mature seed capsules (fruits) were collected from four native populations of open-pollinated plants of *R. vaseyi* in western North Carolina. The first two populations were collected on Pilot Mountain in Transylvania County, and along the ridgeline between Jackson and Transylvania counties. All capsules were pooled and collectively designated Location 1. The second two populations of seeds were collected at mileposts 300 and 305 of the Blue Ridge Parkway in Avery County. Capsules were pooled from these two populations and designated Location 2. The distance between Locations 1 and 2 was 193 km (120 miles).

Following extraction from the capsules, seeds were placed in covered 9-cm (3.5 in) diameter glass petri dishes, each dish containing two pre-soaked germination blotters moistened with tap water. Half the dishes were designated for germination at 25C (77F) and the other half for germination at an 8/16 hr thermoperiod of 30/20C (86/68F). Temperatures within germination chambers varied within ± 0.5C (0.9F) of the set point. The chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400-700 nm) of approximately 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.2 klx). Within each temperature regime, seeds were subjected daily to the following eight photoperiods: 0 (total darkness) ½, 1, 2, 4, 8, 12, or 24 hr. 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. 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, each photoperiod treatment was replicated four times with a replication consisting of a petri dish containing 100 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 ≥ 1 mm (0.04 in) in length. Percent germination was calculated as a mean of four replications per treatment. Data were subjected to analysis of variance procedures (SAS v. 9.1, SAS Inst., Cary, N.C.).

Results and Discussion

Light was required for germination, which is similar to other species of *Rhododendron* L. (rhododendron) (1, 2, 4, 5). Germination increased as a function of photoperiod for each location at both temperatures. Germination was highest at 30/20C (86/68F) for Location 1 (45%) and Location 2 (50%) in the 24 hr photoperiod (Fig. 1). Seeds from Location 2 germinated at higher percentages compared to seeds from Location 1 at both temperatures and all photoperiods. As indicated by a significant interaction between location, temperature, and photoperiod (ANOVA not presented), however, the magnitude of difference between locations was not consistent for each temperature at all photoperiods (Fig. 1). For example, at 30/20C

(86/68F), the difference in germination between locations for 8 hr was 22% (37% minus 15%), whereas for 24 hr the difference was 5% (50% minus 45%). At 25C (77F), the difference between locations was 10% (13% minus 3%) and 5% (30% minus 25%) for photoperiods of 8 and 24 hr, respectively (77F) (Fig. 1).

The alternating temperatures of 30/20C (86/68F) partly compensated for the light requirement at shorter photoperiods. The extent of compensation depended on the location and the photoperiod. The difference in germination percentages between both temperatures for Location 1 at the 4 hr photoperiod was 1% (3% minus 2%) and for Location 2 was 10% (15% minus 5%) (Fig. 1). At the 8 hr photoperiod, the difference between germination temperatures was 12% for Location 1 (15% minus 3%) and 25% for Location 2 (38% minus 13%). Although seeds from Location 2 germinated at slightly higher percentages than seeds from Location 1 at 24 hr, the response of both locations to temperature and photoperiod was similar. The difference in germination may have been due to more vigorous seeds from Location 2 (5).

Effects of light and temperature on seed germination of *R. vaseyi* were very similar to a previous report for seeds from Location 1 (6) and other *Rhododendron* sp. (1, 2, 4, 5). However, the overall germination percentages for *R. vaseyi* were much lower than other *Rhododendron* sp. It is possible seed viability of *R. vaseyi* may be inherently low and methods to determine viability prior to germination would enhance the response. It is also possible there are other barriers to germination, in addition to the light requirement, that require further investigation. Additionally, germination tests of other North Carolina populations of *R. vaseyi* as well as closely related species, might indicate if low seed viability is widespread in the species.

A journal manuscript dealing with this research is in preparation.

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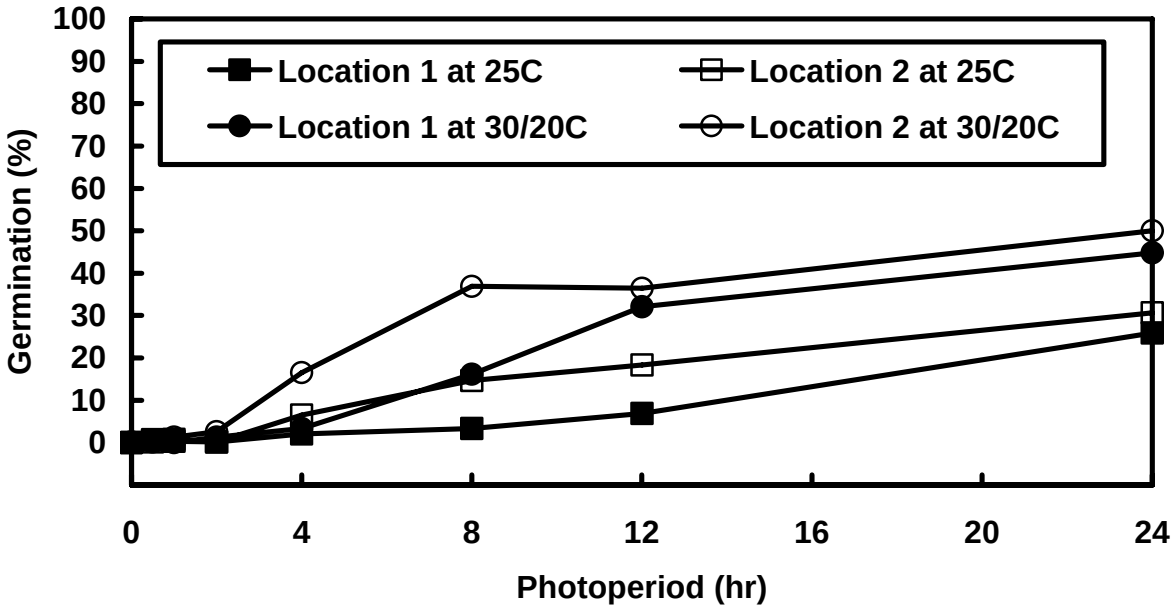

Fig. 1. Cumulative (30-day) germination of seeds of *R. vaseyi* collected from two locations in western North Carolina and germinated at 25C (77F) or an 8/16 hr thermoperiod of 30/20 (86/68C) with daily photoperiods at each temperature of 0 (total darkness), ½, 1, 2, 4, 8, 12, or 24 hr. Symbols represents mean percentage germination of four petri dishes each containing 100 seeds.

ABA-Induced Chilling Resistance in Watermelon

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Introduction

Chilling injury occurs in spring-planted watermelon when temperature reaches 1°C. Exposure of seedlings to low temperatures retards growth, delays flowering, reduces total yields and quality, and even kills the plants (Korkmaz and Dufault, 2001). The objective is to determine whether hormones can protect seedlings from injury. Tests were run in the phytotron using watermelons grown at 26°C day/22°C night temperatures. Seedlings at the first true leaf stage were moved to a B chamber for 36 hr at 4°C with full light. Seedlings were returned to the A chamber, and then rated for chilling injury after 1 week. Individual ratings were summarized as mean and maximum after summarizing over replication and harvest. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

Results and discussion

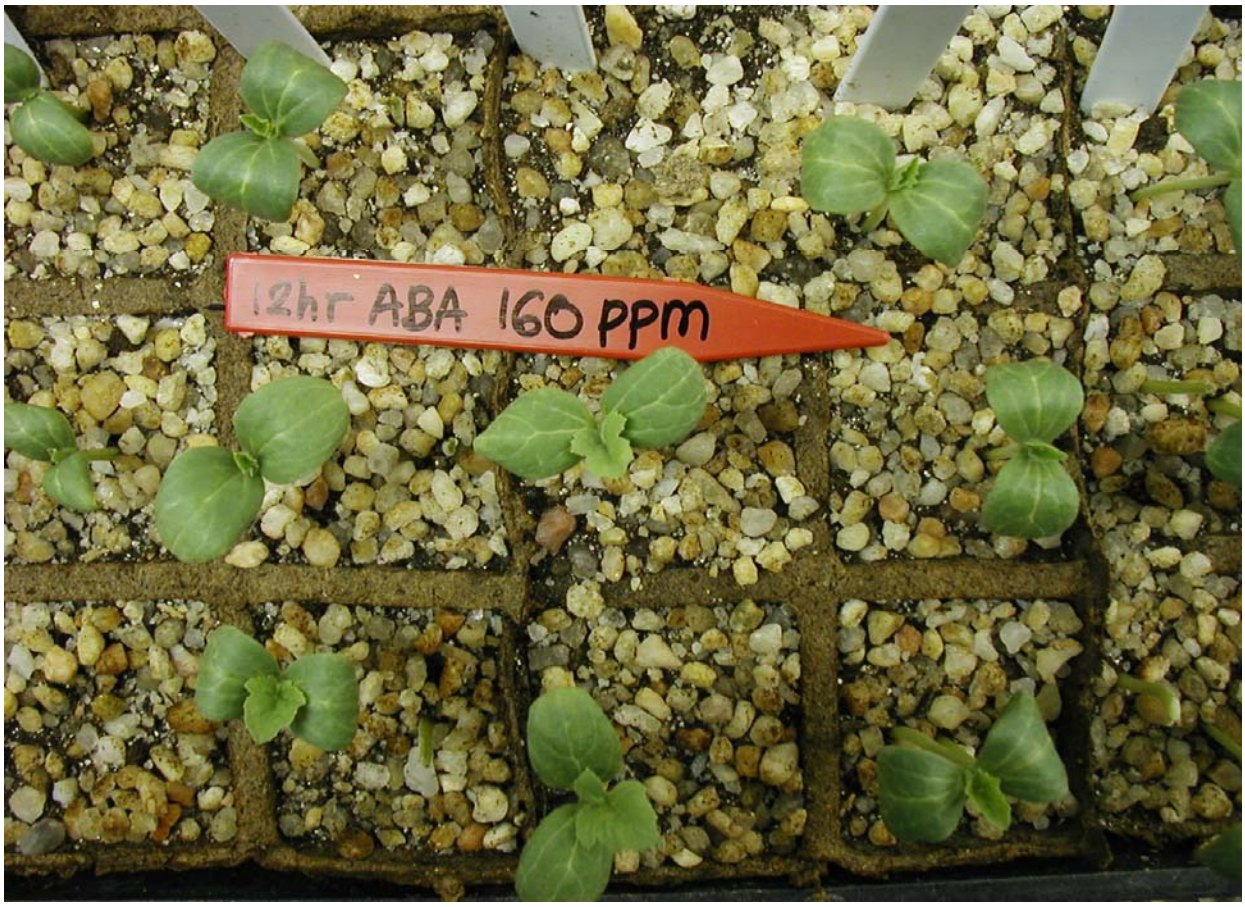
Seedlings treated with ABA at 160 ppm had significantly less injury than the untreated controls. Also, chilling resistant cultivars (Orangeglo) had less injury than susceptible cultivars (Sugar Baby). Hormone treated cultivars that were suceptible had the same injury level as untreated cultivars that were resistant. Of the 9 cultivars tested, Orangeglo and Dixielee were most chilling resistant, and Sugar Baby and Navajo Sweet were most susceptible. ABA treatment had a larger effect on the susceptible plants than on resistant plants (Table 1). The highest concentration of ABA (1280 ppm) caused chlorosis and stunting. In conclusion, 160 ppm ABA sprayed 12 hours before chilling provides protection from damage in watermelon seedlings (Fig 1).

Table 1. Chilling damage ratings for resistant and susceptible watermelons treated with 0 or 160 ppm ABA 12 hours before or after a chilling treatment.[z]

PPM	Cultivar	Mean	Cot	Leaf	Apex
Run 1 (spray before chilling)					
0	Orangeglo	0.3	0.7	0.0	0.0
0	Dixielee	0.5	1.3	0.3	0.0
0	Sugar Baby	2.3	6.4	0.4	0.0
0	Navajo Sweet	2.1	6.3	0.0	0.0
160	Orangeglo	0.4	1.0	0.3	0.0
160	Dixielee	0.2	0.6	0.0	0.0
160	Sugar Baby	0.9	2.8	0.0	0.0
160	Navajo Sweet	1.0	2.9	0.0	0.0
Run 1 (spray after chilling)					
0	Orangeglo	0.7	1.8	0.3	0.0
0	Dixielee	1.3	3.3	0.7	0.0
0	Sugar Baby	2.9	7.7	0.9	0.0
0	Navajo Sweet	2.1	6.1	0.3	0.0
160	Orangeglo	0.3	1.0	0.0	0.0
160	Dixielee	0.5	1.1	0.3	0.0
160	Sugar Baby	1.6	4.6	0.1	0.0
160	Navajo Sweet	1.3	3.8	0.0	0.0
Run 2 (spray before chilling)					
0	Orangeglo	1.0	2.0	1.0	0.0
0	Dixielee	1.0	2.2	0.8	0.0
0	Sugar Baby	3.0	7.6	1.0	0.4
0	Navajo Sweet	2.2	6.3	0.3	0.0
160	Orangeglo	0.1	0.3	0.0	0.0
160	Dixielee	0.7	1.7	0.2	0.1
160	Sugar Baby	0.9	2.8	0.0	0.0
160	Navajo Sweet	1.0	3.1	0.0	0.0
Run 2 (spray after chilling)					
0	Orangeglo	0.3	1.0	0.0	0.0
0	Dixielee	0.5	1.5	0.0	0.0
0	Sugar Baby	2.0	5.8	0.2	0.0
0	Navajo Sweet	1.3	3.8	0.1	0.0
160	Orangeglo	0.1	0.2	0.0	0.0
160	Dixielee	0.4	1.1	0.0	0.0
160	Sugar Baby	2.4	7.0	0.3	0.0
160	Navajo Sweet	2.4	7.0	0.2	0.0
LSD (5%)		0.9	2.1	0.7	0.1

z Damage rated 0-9 on cotyledon, first true leaf, and shoot apex (0=no damage, 9=plant dead). Data are means of 2 replications and 3 ratings. Data for only 4 of 9 cultivars are shown.

Fig. 1. 160 ppm ABA sprayed 12 hours before chilling provides protection from damage in watermelon seedlings.



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Photoperiod and Growth Temperature Effects on Sex Expression in Watermelon

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Introduction

Sex expression and sex ratio in Cucurbitaceae have generally been of considerable interest. Although sex expression in cucumber plants is determined genetically, it can be modified by several environment factors. High nitrogen, short days, low-light intensity, and low night temperature are among the factors which favor femaleness. Reverse conditions tend to cause maleness (Hesllop-Harrison 1957). However the ratio of male to female flowers in the watermelon was greater with 8 hours day than a 16 hours day of 27°C than at 22°C or 32 °C (Rudich and Peles 1976). The objective is to determine whether photoperiod and temperature can effect on the sex expression in watermelon. However, we conducted experiment with four different environmental conditions on the watermelon sex expression. Tests were run with four different growth chambers using watermelon grown at 32/24°C/8h, 32/24°C /16h, 24/16°C/8h, and 24/16°C/16h in the phytotron. Plants were fertilized before seedling with one time per week for low nutrition and seven times per week for high nutrition respectively. Plants were rated the days to first flowering, number of female and male flowers, and vine length to determine the photoperiod and temperature effect on the sex expression. Individual ratings were summarized as mean and maximum after summarizing over replication and harvest. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

Results and discussion

Low temperature and long-day induced more female flowers than male flowers in watermelon plants while day length and fertilization with high temperature were not significantly difference effect on the sex expression (Table 1). Fertilization was not effect on the sex expression but the

vine length with low temperature (Table 2). In conclusion, temperature had a great effect and other factors were not directly effect on the sex expression in watermelon.

Table 1. Regardless of fertilization, the percentage female and male flowers for watermelons treated with four different environmental conditions.

Chamber	VNLN	PCFM	PCMM	PCF1	PCF2	PCF3	PCF4	PCM1	PCM2	PCM3	PCM4
24C-16h	1.6	21	79	19	21	21	22	81	79	79	78
24C-8h	1.8	14	86	10	14	16	17	90	86	84	83
32C-16h	1.3	15	85	11	15	17	17	89	85	83	83
32C-8h	2.8	16	84	13	17	17	18	87	83	83	82

z Percentage of female and male nodes.

Table 2. Percentage female and male flowers for watermelons treated with four different environmental conditions.

Chamber	Fertilizer	VNLN	PCFM	PCMM
24C-16h	High	2.1	18	82
24C-8h	High	2.4	11	89
24C-16h	Low	1.0	23	77
24C-8h	Low	1.3	18	82
32C-16h	High	1.4	16	84
32C-8h	High	3.4	15	85
32C-16h	Low	1.2	15	85
32C-8h	Low	2.3	18	82

z Percentage of female and male nodes.

Fig. 1. Watermelon grown at 32/24°C/8h (B3 chamber), and 32/24°C /16h (B4 chamber).

Fig. 2. Watermelon grown at 24/16°C/8h (B7 chamber), and 24/16°C/16h (B8 chamber).

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Effect of High Temperature on Extreme Substrate Acidification by Geranium (*Pelargonium x hortorum* Bailey).

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Abstract

The cause of sudden substrate pH decline by geranium is unknown and previous reports suggest it may be due to high temperature. The first of 2 experiments compared plants grown at 4 temperatures (14/10, 18/14, 22/18 and 26/22º C day/night). With increasing increments of temperature, substrate pH declined from 6.8 to 4.6 at 63 d. Low tissue P has also been shown to cause plants to acidify. In the first experiment, tissue P was deficient (below 0.2) in the 3 highest temperature treatments and it was unclear whether the cause of substrate acidification was due to P deficiency and/or high temperature. The second experiment was a factorial of 3 temperatures (18/14, 22/18 and 26/22º C day/night) by 5 pre-plant P rates [0, 0.33, 0.67, 1.33, 2.67 g triple superphosphate (TSP) · L^{-1} substrate]. At 28 d tissue P was adequate in plants at all temperature and P treatments except 0 TSP. Yet, pH was lower in the high temperature treatment with all P rates except the highest, indicating that temperature acts independent of tissue P level. At 63 d in the 0.33 and 0.67 TSP treatment, tissue P was deficient but not significantly different and pH decreased with increasing temperature from 5.6 to 4.8 and 5.9 to 4.7, respectively. At the 1.33 TSP treatment tissue P is adequate at low temperature and deficient at the two higher temperatures yet pH decrease as temperature increase. In the highest P treatment all tissue P levels are adequate and pH declines with each increase in temperature. Again, the 63 day results show that temperature is acting independent of tissue P. When plants received P, pH fell to below 5.2 by 57 d when temperature was high. These data indicate high temperature stressed geraniums increase the rate of acidification, which offers one explanation for sudden substrate pH decline.

Effect of Phosphorus Deficiency and High Temperature on Ammonium and Nitrate Uptake by Geranium (*Pelargonium x hortorum* Bailey).

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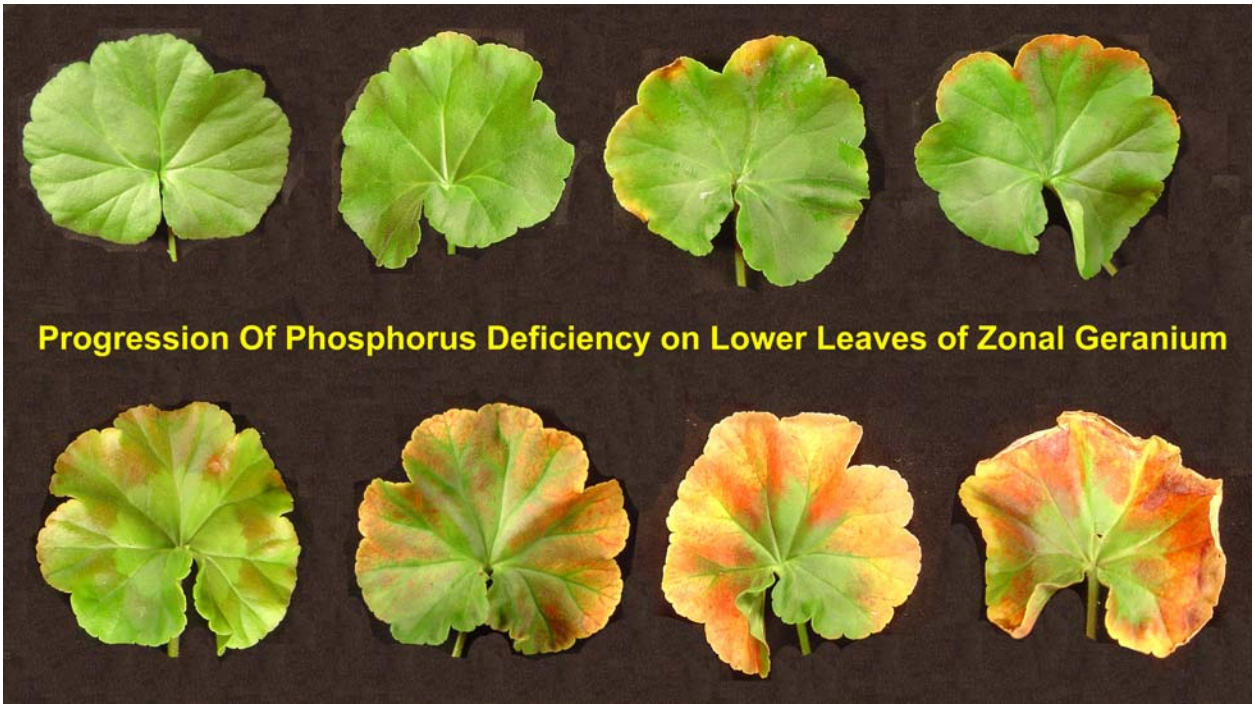
Abstract

The cause of sudden substrate pH decline by geranium is unknown and may be due to a shift in cation-anion balance. Nitrogen plays a very important role in cation-anion balance since it accounts for over 50% of the mineral ions that will cross the plasma membrane and is the only mineral nutrient that can be absorbed as a cation (NH_4^+) or anion (NO_3^-). Studies have shown that P deficiency will suppress NO_3^- uptake or suppress NO_3^- uptake and increase NH_4^+ uptake. These changes cause a shift in the cation-anion uptake ratio and if favored towards NH_4^+ would cause acidification of the rhizosphere. This experiment compared geraniums (*Pelargonium x hortorum* Bailey 'Designer Dark Red') grown in 200 L hydroponic tanks at 2 temperatures (18/22 and 22/26° C night/day) and with or without P. 3, 11, and 19 days after transplanting (DAT), plants were placed into an identical solution contain either $^{15}NH_4^+$ or $^{15}NO_3^-$ for 24 hours. Influx of $^{15}NH_4^+$ was not significantly affected by temperature and was suppressed by P starvation 19 DAT. Influx of $^{15}NO_3^-$ was not significantly affected by temperature and was suppressed by P starvation 11 and 19 DAT. At the control temperature treatment the $^{15}NH_4^+$:$^{15}NO_3^-$ influx ratio increased significantly from 0.34 to 0.46 and 0.30 to 0.38 when plants did not receive P at 11 and 19 DAT, respectively. This suggests an increased acidification rate by P starved plants. pH was maintained at 5.8 throughout the experiment and tanks with plants

receiving P consumed less than half the mEq of titrating base per gram dry weight plant than tanks with plants that were devoid of P. Data indicate P stressed geraniums suppress uptake of N (primarily NO_3^-) and increase the rate of acidification, which offers one explanation for sudden substrate pH decline.



Ecological and Physiological Basis for the Distribution of Woody Plants Along Water Availability Gradients in the Eastern United States Mixed Forest (Study 2)

Pamela P. Abit and William A. Hoffmann

Introduction

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

The distribution of species in eastern forests suggests that water availability does exert a strong control on the success of woody plants. Xeric forest is typically dominated by drought-tolerant species like the white, red and black oak canopies. On the other hand, mesic forest is dominated by stable maple-basswood forest. Some species are known 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), the occurrence of woody plant species is largely controlled by water availability. Water, an important resource for plants, affects plant physiology and growth. 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 gradients may be influenced by their physiological responses to environmental conditions (Barbour et al., 1999).

Although Eastern US forests are not considered to be water-limited ecosystems, water relations and other physiological filters that determine the species composition in a particular site, such as nutrient availability, 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 three physiological filters, which include seed germination, early seedling growth and survival. We compared the ability of mesic and xeric tree species to germinate, grow and survive under varying levels of water stress.

Materials and Method

Study Species

Congeneric species pairs of *Quercus, Vaccinium, Fraxinus,* and *Nyssa* were used. Congeneric species pairs were used to ensure phylogenetic independence, which is an important consideration for making inferences in comparative studies and improving the statistical power of comparison between two groups (Ackerly, 1999). In each pair, one species was adapted to xeric conditions, while another was adapted to mesic conditions.

Germination experiment

This experiment was performed to compare the ability of mesic and xeric species to germinate under water stress. Germination of the *Fraxinus* species pair was done in the previous year (Abit and Hoffmann, 2005). Prior to germination, the seeds of *Quercus alba*, *Q. nigra, Vaccinium stamineum, V. corymbosum, Nyssa sylvatica,* and *N. aquatica* were warm and cold stratified in pre-soaked blotter in petri dishes to break their dormancy. Prior to setting up the germination experiment, the seeds were surface 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 soil 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 for each species mentioned above. The Petri dishes containing the seeds were placed in a growth chamber and maintained at 30 ^{o}C (day) and 20 ^{o}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.

Osmotic adjustment

Measurements were taken from the apical 10 mm of primary root tips of seedlings that were grown in substrate of different water potentials (0 MPa, -0.2 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa). Harvested roots were rehydrated for 10 minutes and were frozen at -20 ^{o}C. After 2 days, the frozen roots were thawed at room temperature and were centrifuged at 10,000 rpm for 20 minutes. Ten µl of cell sap from each root tip was collected and placed on a filter paper disc. Osmotic potential readings were then taken using the VAPRO vapor pressure osmometer model 5520 (Wescor Inc., Logan UT.). Measurements were replicated 10 times per species. Osmotic adjustment (OA) is calculated as the difference between osmotic potential of roots at full turgor between the control and stressed plants.

$OA = \psi_{\pi}(100)$ control - $\psi_{\pi}(100)$ stressed seedling

Where: $\psi_{\pi}(100)$ = osmotic potential at full turgor

Dry-down experiment

This experiment compared the ability of mesic and xeric species to survive extreme water stress by evaluating survival after re-watering subsequent to drought exposure. When *Nyssa* 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, which corresponds to the different leaf water potentials.

Nyssa sylvatica

a b c d e f

Nyssa aquatica

Figure 1. Wilt stages of *N. sylvatica* and *N. aquatica,* respectively: a) normal, b) slightly wilted, c) wilted d) severely wilted, e) nearly dead, and f) presumed dead.

Results and Discussion

Figure 3 shows that germination rate decreased with decrease in substrate water potential. Germination of xeric *N. sylvatica* was significantly different to the mesic species, *N. aquatica* (P=0.0078) but the germination rates between *N. sylvatica* and *N. aquatica* were not significantly affected by water stress. On the other hand, the germination of xeric species of *V. stamineum* and *Q. alba* were significantly higher than *V. coymbosum* and *Q. nigra*, respectively, across different levels water stress (P<0.0001 and P=0.0267, respectively). The low germination rate at low water potentials could be a strategy of xeric *Nyssa* to avoid water stress. In contrast, desiccation tolerance could be the drought resistance strategy that xeric *V. stamineum* and *Q. alba* adapt, which enables them to germinate even under water stress. These results suggest that the ability to germinate under water stress may depend on the drought resistance strategy that the species adapts and that germination is not be a strong determinant for the observed distribution of the species.

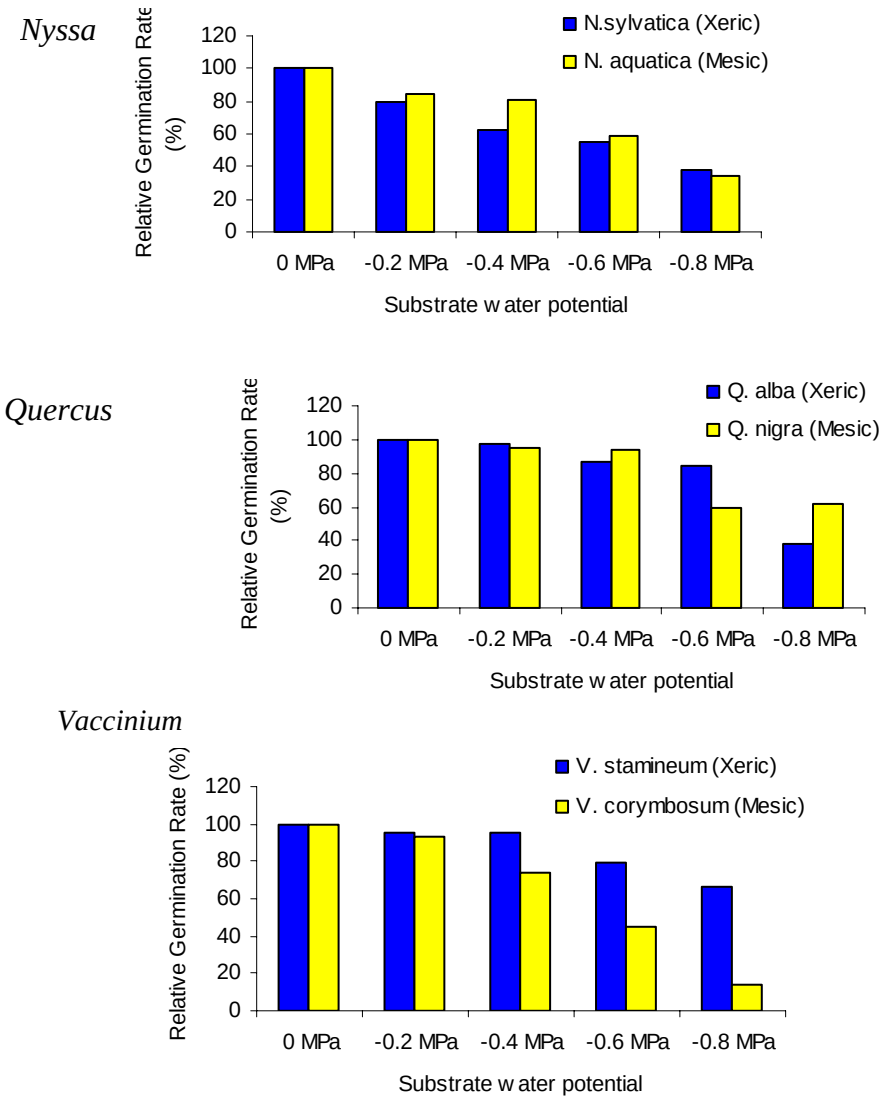


Figure 3. Percent germination of *Nyssa*, *Quercus,* and *Vaccinium* grown on substrate with different water potential levels.

The ability to maintain cell turgor and thus water uptake under increasingly water stressed conditions by active accumulation of solutes in the cell sap is known as osmotic adjustment (OA). The osmotic adjustment in roots of mesic species, *F. pennsylvanica* and *N. aquatica*, were not significantly different from the roots of xeric *F. americana* and *N. aquatica*, respectively (Figure 4). On the other hand, xeric *Q. alba* had a significantly higher osmotic adjustment than the mesic *Q. nigra* up to -0.4 MPa substrate water potential (p=0.0360). The higher degree of OA in *Q. alba* is also significantly correlated with the high germination rates in

water-stressed conditions (P=0.0003, data not shown). However, the ability to osmotically adjust under water stress is not a trend observed among all xeric species tested.

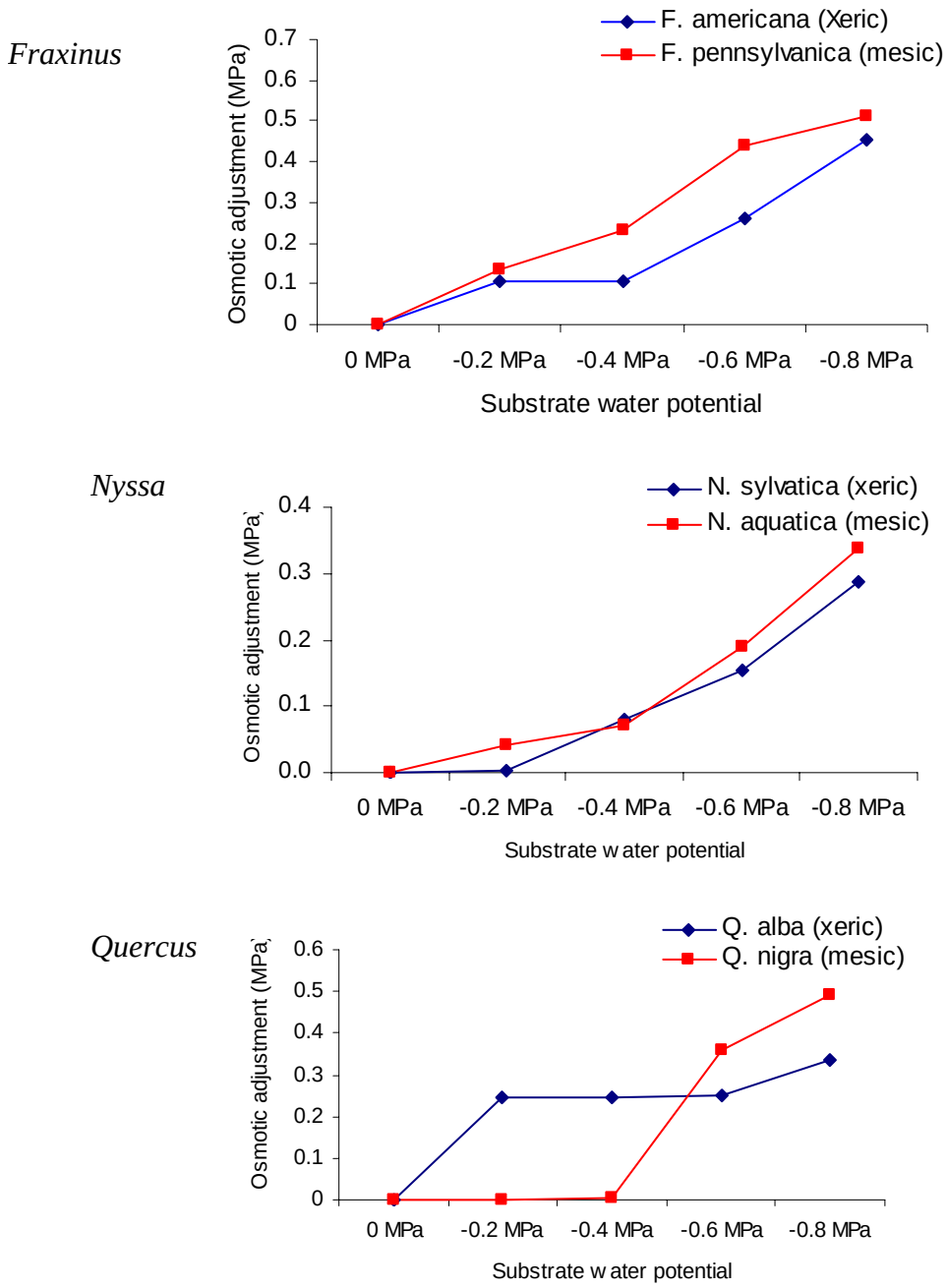


Figure 4. Osmotic adjustment in *Fraxinus, Nyssa,* and *Quercus.*

The differential responses of seed germination, seedling growth, and establishment to water stress often determine the distribution of species across a landscape. Seeds may germinate but drought continues to act as a filter to plant survival after germination and initial seedling

establishment. The dry-down experiment evaluated the drought tolerance of the xeric and the mesic *Nyssa* species. The different wilt stages in *N. sylvatica* and *N. aquatica* closely corresponded to different leaf water potentials. The leaf water potential of seedlings decreased linearly with wilt stages (data not shown). Figure 1 shows the observed wilt manifestation such as necrosis. Figure 5a reveals that *N. sylvatica* had a higher complete stem survival than *N. aquatica*, and there was no significant difference in partial stem survival under water stress. The results reveal that seedlings of *N. aquatica* are more vulnerable to wilting than *N. sylvatica* at extreme water stress. *N. sylvatica* is capable of higher survival under water stress, and thus is more adapted to dry habitats.

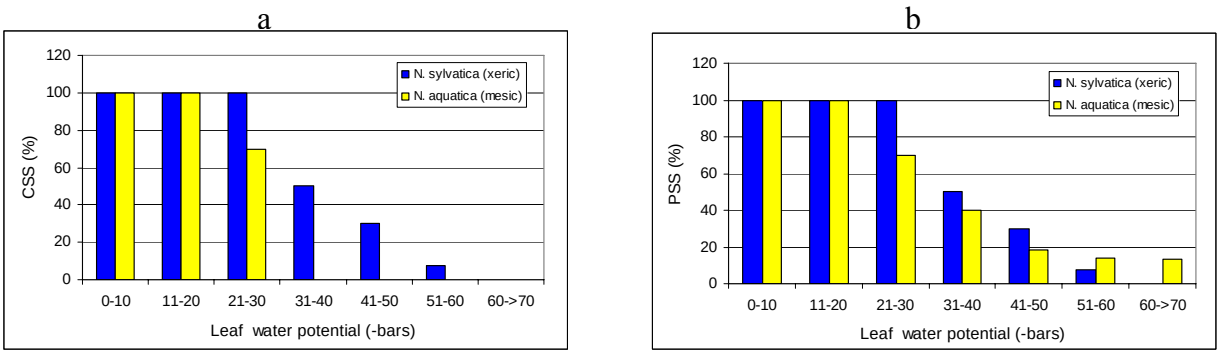


Figure 5. Percent complete stem survival (CSS) (a) and percent partial stem survival (PSS) (b) of *N. sylvatica* and *N. aquatica* after dry-down exposure.

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Wound-Induced Gene Expression Tomato

Eric Davies

We are continuing our work trying to determine the array of systemic signals evoked by wounding that are capable of eliciting gene expression (transcript accumulation) in distant (non-wounded) leaves of tomato plants. In our most recent publication (Vian and Davies, 2006) we used a heat-wound to evoke a variation potential (VP), leaf excision (cut-wound) to evoke an action potential (AP) and a heat-wound followed 90 seconds later by excision of the heat-wounded leaf, and in all cases measured accumulation of the transcript encoding a chloroplast mRNA binding protein (CMBP). The results are consistent with the hypothesis that the two different electrical signals (VP and AP) are both capable of eliciting CMBP mRNA accumulation, but with different kinetics, and, since their effects are not additive, they presumably share some common aspects.

A. Vian, E. Davies 2006 Two different wound signals evoke very rapid, systemic CMBP transcript accumulation in tomato. Plant Signaling and Behavior 1 (5) 261-264.

Regulation of leaf area index under drought in a tropical pasture grass.

William Hoffmann & Renee Marchin
Department of Plant Biology

Leaf phenology is a fundamental process influencing net primary productivity, ecosystem water and energy balance and vegetation flammability. A realistic representation of leaf dynamics in ecosystem models is essential for reliable projections of climate change effects on ecosystems. Unfortunately, unlike many other processes, our ability to model the response of leaf dynamics to environmental change is rudimentary. In this study, we are focusing on the response of leaf senescence to drought stress in *Brachiaria brizantha*, a widely planted C4 pasture grass in the tropics, with an interest in generating a robust model for predicting the effect of seasonal drought on flammability of tropical grasslands, savannas, and pastures.

Methods

We grew *Brachiaria brizantha* from seed and transplanted the seedlings into 30cm deep pots in a soil composed of 90% clay and 5% peat and 5% sand by volume.

Prior to any experimental treatments, we performed a suite of physiological measurements to provide data for modeling photosynthesis and water relations. Photosynthesis of a subset of individuals were was measured with a LICOR 6400 photosynthesis system over a range of CO_2 and light intensities. Hydraulic conductance of leaves, roots, and whole plants was measured using the evaporative flux method (Sack *et al.*, 2002). The effect of leaf water potential on stomatal conductance was determined by performing concurrent measurements of leaf water potential and gas exchange.

We produced a model of whole-plant water and carbon balance. Carbon relations were simulated using a biochemical model of C4 photosynthesis (von Caemmerer, 2000). Water relations are represented by stomatal, leaf, and root conductances. Stomatal conductance in the model responds to water stress via a feedback loop involving leaf water potential. We used this model to make predictions of patterns of leaf senescence that should maximize whole-plant carbon gain.

The next step of this project is to implement watering and thinning treatments in a factorial experiment. The four water treatments will include a well-watered control and three treatments in which we will gradually induce water stress by gradual restriction of daily water addition. The thinning treatments will include a control, 33% reduction in leaf area, and 67% reduction in leaf area. Measurements of leaf senescence, stomatal conductance, photosynthesis, and leaf water potential will be obtained during the course of these treatments.

Results and discussion

Photosynthetic response to light and CO_2 show patterns typical of C4 grasses, such as low CO_2 saturation point and high light saturation point (Figure 1). The biochemical model of C4 photosynthesis (von Caemmerer, 2000) fit reasonable well to the data, but exhibits an overly abrupt transition from the initial CO_2-limited portion of the curve to the CO_2 saturated portion (Figure 1).

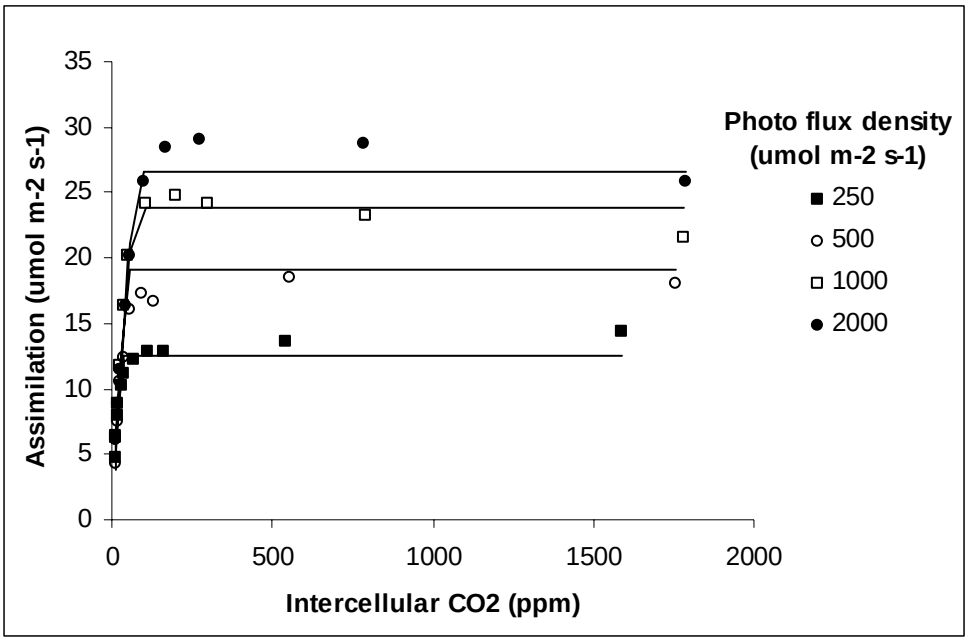

Figure 1. Example of measured and modeled rates of photosynthesis under varying CO_2 and light intensity. The lines represent predicted rates of photosynthesis using a biochemical model of C4 photosynthesis (von Caemmerer, 2000) fit to the observations.

Stomatal conductance was positively correlated to leaf water potential. Since stomata respond strongly to other factors, such as light and relative humidity, there was considerable scatter in this relationship (Figure 2). However, for modeling stomatal response to leaf water

potential, we are interested in the relationship between water potential and maximum conductance, as approximated by the curve in Figure 2. Leaf hydraulic conductance was 0.958 ±.086 mmol m^{-2} s^{-1}. Root hydraulic conductance was 2.55 ± 0.36 mmol m^{-2} s^{-1}.

We used our model of carbon and water relations to predict carbon gain for plants of different leaf areas under a range of water supply rates (Figure 3). In addition to the data described above, this required information on soil physical properties, which were obtained by the Soil Physics Laboratory of the Department of Soil Science. Although this model is preliminary, and requires several improvements, these simulations suggest how water availability influences the optimal leaf area that should be maintained if carbon gain is to be maximized. The model predicts that leaf area should be maintained near the inflection point of the curve for the particular rate of water supply. These predictions provide a testable hypothesis of how leaf area should change under drought stress. If grasses do behave optimally, this will provide useful approach for modeling canopy dynamics of grass-dominated ecosystems. More likely, plants will not exhibit a perfectly optimal strategy, but the results of this experiment should nevertheless provide useful insight for modeling grass canopies.

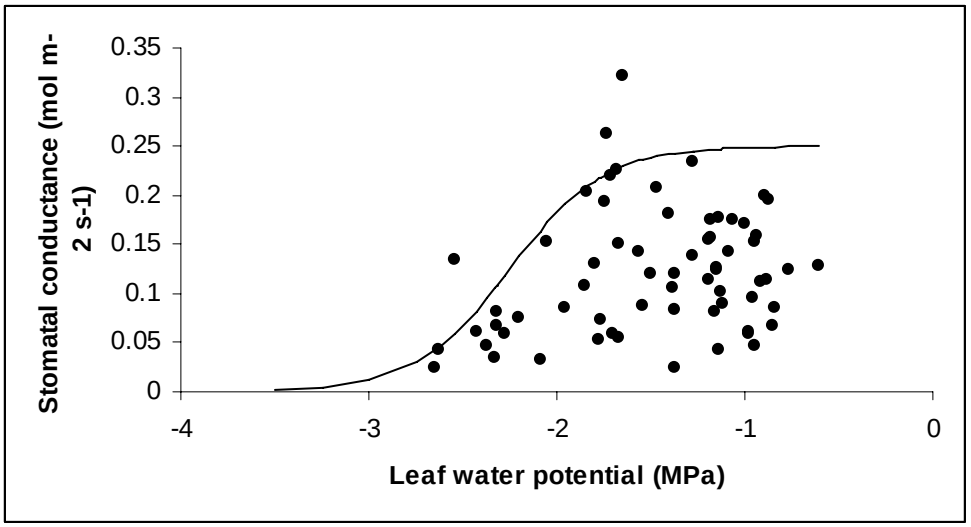

Figure 2. The relationship between leaf water potential and stomatal conductance. The line shows the relationship used to model the relationship between maximum stomatal conductance and leaf water potential.

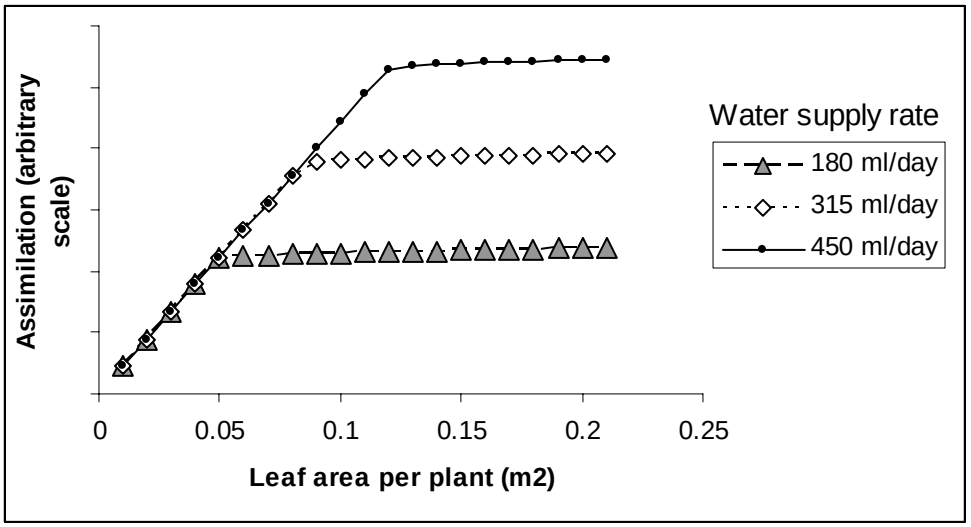

Figure 3. Predicted relationship between leaf area and daily integrated photosynthesis for three water supply rates.

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Increasing Drought Tolerance in Tomato by Metabolic Engineering of the Phosphoinositide Pathway

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Introduction:

Plants sense a wide range of environmental signals and respond with changes in gene expression and growth. The distinction of those different signals occurs through specific receptors. Signal transduction and amplification through second messengers (e. g. Ca^{2+}, $InsP_3$) mediates the response. The phosphoinositide pathway is involved in plant responses to salt, drought and cold stress (Meijer and Munnik, 2003). Our objective is to increase stress tolerance in crop plants (tomato) by regulating intracellular levels of inositol-1,4,5-trisphosphate ($InsP_3$). Expression of the human type I inositol polyphosphate 5-phosphatase (InsP 5-ptase) has been shown to significantly reduce the $InsP_3$ level in transgenic *Arabidopsis* lines. These transgenic *Arabidopsis* plants exhibit a delayed and reduced response to gravity, as well as increased tolerance to drought stress (Perera et al., 2006; Perera et al, unpublished data). We therefore transformed tomato cotyledons (cv. Micro-Tom) with InsP 5-ptase. Twenty transgenic tomato lines and two empty vector (control) lines were established and high levels of expression of the transgene was detected in the transgenic tomato lines by RT-PCR. The InsP 5-ptase expressing transgenic plants were tested for tolerance to water deficit stress and analyzed for morphological changes by using growth chambers of Phytotron, NCSU.

Results:

I. Expression of InsP 5-ptase in tomato resulted in increasing of drought tolerance.

It was observed that transgenic tomato plants expressing InsP 5-ptase were more drought tolerant and survived longer than the wild type controls when water was withheld (Fig. 1). The rate of water loss from detached leaves of transgenic plants is decreased by 20% compared to the wild

type plants (data not shown) suggesting that the regulation of stomata may be different in the transgenic plants.

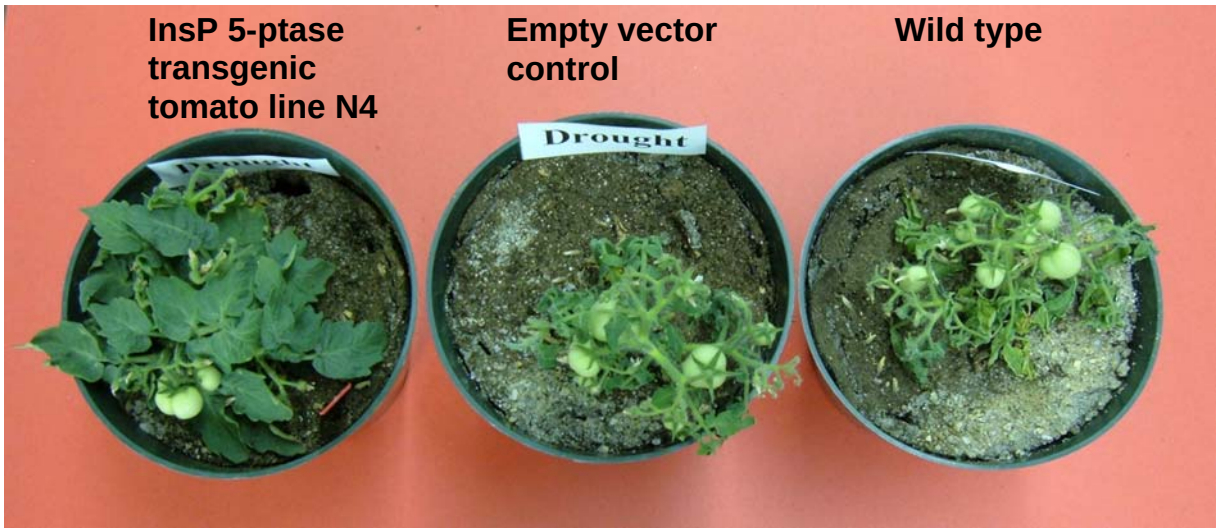

Figure 1. InsP 5-ptase expressing tomato lines (generation T_1) showed no sign of wilting or visible stress responses after 3 weeks of drought stress compared to wild type and vector control lines.

II. InsP 5-ptase transgenic tomato lines showed range of morphological changes

To understand the mechanisms involved in stress tolerance of these transgenic tomato plants we characterized morphological and physiological parameters of independent homozygeous transgenic lines in comparison with wild type and vector control lines. Leaf morphology was substantially different in InsP 5-ptase transgenic tomato lines compare with both control lines (wild type and vector control). We confirmed that transgenic leaves were thicker and had bigger cells on leaf surface then control leaves (Fig. 2; Table 1). Average leaf area and stem diameter were also significantly higher in transgenic leaves (Table 1).

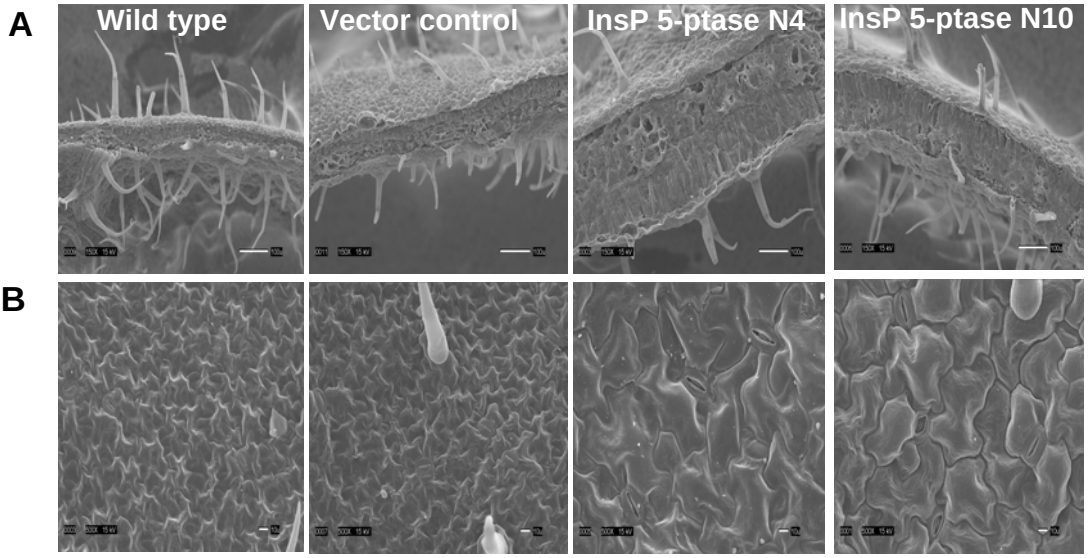

Figure 2. Scanning electron microscopy of leaf cross section (A) and leaf surface (B) of wild type, vector control and two transgenic lines expressing InsP 5-ptase gene.

Table 1. Leaf and stem morphological characteristics of wild-type, empty vector line (control) and transgenic InsP 5-ptase tomato lines.

Genetic line	Average area per leaf (cm2) ± SE	Specific leaf area (SLA) (mm2/mg) ±SE	Average stem diameter (mm) ±SE
Wild type (control 1)	6.0 ± 0.1	2.4 ± 0.13	4.53 ± 0.29
Empty vector (control 2)	5.59 ± 0.28	2.29 ± 0.19	4.81 ± 0.12
InsP 5-ptase N6	10.84 ± 0.95	1.35 ± 0.15	7.37 ± 0.32
InsP 5-ptase N4	12.73 ± 0.53	1.42 ± 0.25	8.15 ± 0.42
InsP 5-ptase N7	10.17 ± 1.53	1.57 ± 0.23	6.58 ± 0.14
InsP 5-ptase N2	9.36 ± 0.66	1.91± 0.31	4.59 ± 0.25
InsP 5-ptase N5	11.05 ± 0.20	1.63 ± 0.36	5.1 ± 0.28
InsP 5-ptase N1	10.40 ± 0.42	1.58 ± 0.29	6.53 ± 0.47

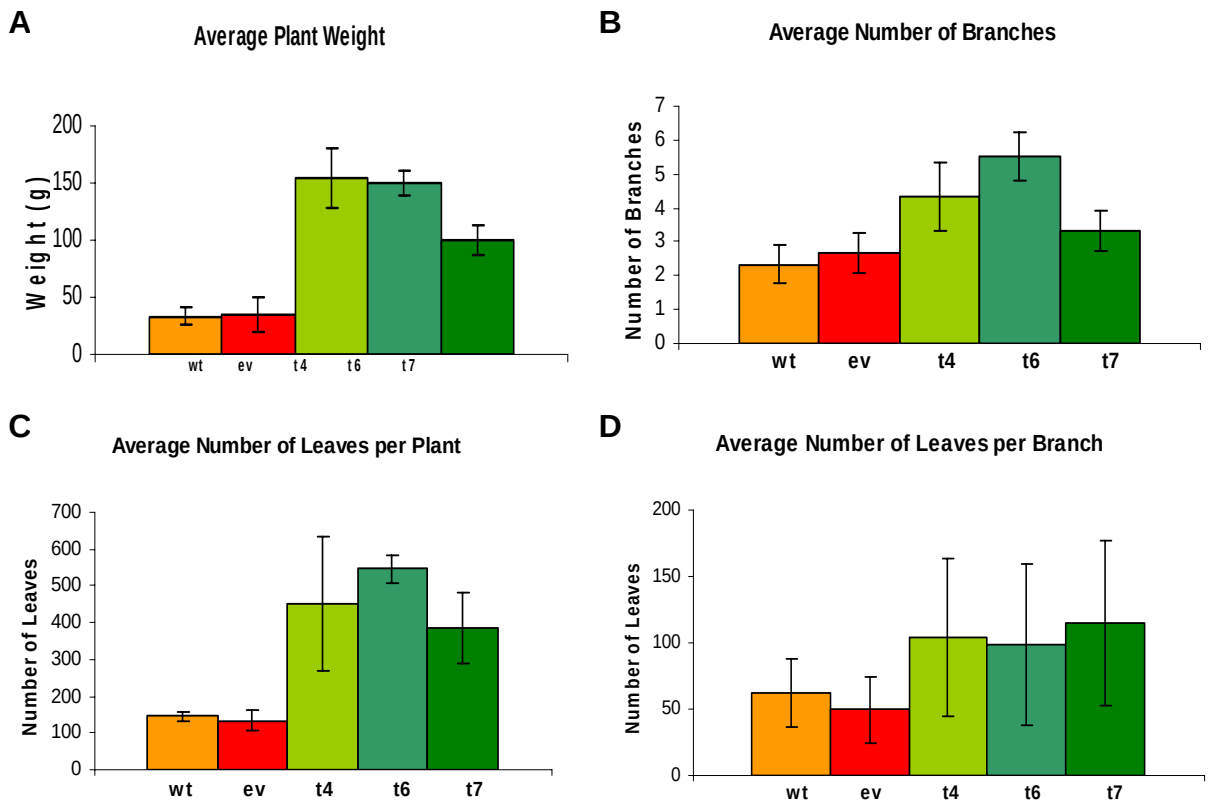

Figure 3. Plant weight (A), number of branches (B), and number of leaves (C,D) observed in wild type, empty vector control, and three tomato different transgenic lines (N4,6,7) expressing the InsP 5-ptase gene.

We found also that the expression of InsP 5-ptase in tomato caused significant changes in plant shape such as increased plant weight, branching and number of leaves (Fig. 3).

Conclusion:

The significant increase of water deficit stress resistance was observed in transgenic InsP 5-ptase tomato lines. Results of our drought stress experiments confirmed data generated early on *Arabidopsis* plants expressed same gene (Perera et al., 2006, unpublished data). We found also that the expression of InsP 5-ptase in tomato caused significant morphological changes such as increased plant weight, branching, diameter of stem, number of leaves, leaf thickness and leaf area. The mechanism behind increased of stress tolerance from manipulations of $InsP_3$ levels in plant cells remains an open question. However, we suggest that increasing of leaf thickness can have a positive impact on the plant performance under a water deficit stress.

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Maize Transformation

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Plant Biology Department

One of the objectives of our project is to investigate the effects of RB7 MARs on transgene silencing in maize. We transformed immature embryos of Hi II hybrid (done in collaboration with the Plant Transformation Center of Iowa State University) by *Agrobacterium* - mediated method. We used a set of binary vectors containing the *ubiquitin*-intron promoter to drive the plant selectable marker, *bar*, which confers resistance to the herbicide bialaphos. The red-shifted *gfp* reporter gene was driven by either CaMV 35S promoter or the rice actin promoter. RB7 MARs sequences flanked the reporter cassette and their effects on reporter gene expression will be assessed in comparison to the same cassette without MARs. . The immature embryos of Hi II were infected with *Agrobacterium tumefaciens* strain EHA101 carrying these binary vectors. Callus clones and regenerated plantlets were sent to NCSU for GFP analysis and T1 seed production through back crossing to B73 and to A188 was done by Iowa State U and North Carolina State U, respectively. The transgenic T0 plants were grown to maturity in a walk-in A chamber and back crossed to wild type A188 plants that were also grown in another walk-in A chamber in the Phytotron. Transgene (GFP) expression in T1 backcross seed has been evaluated in the +MAR and the –MAR lines for both promoters.

All lines of transgenic callus were collected for *gfp* protein analysis. The protein quantity was determined by the Bradford method. The *gfp* fluorescence was measured with QPCR machine (Stratagene) using the plate reader function. A standard curve was created using a standard GFP (#632502, Clontech). We observed a significant difference in *gfp* expression measured as picogram of GFP per μg extracted protein, in callus lines transformed with two different promoters. The overall expression of the transgene driven by CaMV 35S promoter was several fold (5-7x) higher than that from rice actin promoter. However, MARs did not seem to have any effect on the expression of the transgene driven by either promoter. In fact, we noticed a lower expression from CaMV 35S in the MAR lines than that in non-MAR lines, and there was no MAR effect on the expression from actin promoter. The rice actin promoter has been reported

as a strong constitutive promoter driving the expression of transgenes in monocots (McEnroy et al., 1991 Mol. Gen. Genet. 231:150-160). Significant increase in the transient expression of *gus* (Chibba et al., 1993 Plant Cell Rep. 12:506-509) and stable expression of *gfp* (Cho et al., 2002 Phygiol. Plant. 115:144-154) driven by rice actin promoter were reported in barley even though the expression of *gfp* was gradually lost in T0 and later generations (T1 and T2) progeny. However, in our maize transformation experiments, both by particle bombardment (last year's report) and by the *Agrobacterium* method we report this year, we observed significantly higher expression of *gfp* driven by the CaMV 35S promoter, relative to the same transgene driven by rice actin promoters. The consistently higher expression from CaMV 35S promoter than rice actin promoter is probably due to the high transcription rate from the strong promoters that might trigger PTGS. Flanking MARs may be effective in reducing trangene silencing caused by TGS, but may have little or no effect on PTGS. Experiments are being conducted in our lab to understand the mechanistic function of RB7 MARs.

Several lines of T1 backcross from the four constructs to the wild type B73 were visually scored for *gfp* expression in 3-4 days old etiolated seedlings with a scale from 1-3 (1- being lowest and 3- being highest expression). We did not observe a significant difference in *gfp* expression level among the MAR and non-MAR lines. More T1 lines will be screened for a complete evaluation.

Fungicide Resistance And Pathotype Determination Of *Pseudoperonospora cubensis*, Causal Agent Of Cucurbit Downy Mildew

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Dept. Plant Pathology, North Carolina State University

Downy mildew is one of the most significant foliar diseases of cucurbits. The disease threatens production of cucurbits worldwide (4). Even though downy mildew is not a new disease, it has become the most important disease of cucumber since 2004. In that year, the North Carolina cucumber crop experienced the most severe epidemic of downy mildew on record. Yield losses averaged 16 million dollars statewide. In 2005 and 2006, the North American Plant Disease Forecast Center received reports from 17 states from Florida to Michigan, as well as Ontario and Quebec, Canada (2).

The causal agent of cucurbit downy mildew is *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovtsev, an obligate parasite that causes disease in some members of the Cucurbitaceae. When a pathogen shows differing degrees of host specificity within a family of plants, it is called a pathotype (1). There were five pathotypes initially determined in 1987 (Table 1) (4, 5). However, in light of the recent epidemic a new study is underway to determine which pathotypes are present today. There is at least one other similar study underway in the Czech Republic (3).

In addition, field fungicide evaluation trials in 2004 revealed that the contemporary population of *P. cubensis*, might be resistant to the mefenoxam and stobilurin fungicides. Similar results were found in North Carolina field trials in 2005 and 2006, as well as in other areas of the United States. In response to this phenomenon, fungicide resistance assays have been underway in the North Carolina State Phytotoron.

Table 1. Pathotype designations based on *Pseudoperonospora cubensis* and host compatibility (4).

Host	Pathotype				
	1	2	3	4	5
Cucumis sativus	+	+	+	+	+
C. melo var. *reticulatus*	+	+	+	+	+
C. melo var. *conomon*	--	+	+	+	+
C. melo var. *acidulous*	--	--	+	+	+
Citrullus lanatus	--	--	--	+	+
Cucurbita spp.	--	--	--	--	+

+ Highly compatible host interaction, -- incompatible or very slightly compatible host-pathogen interaction

MATERIALS AND METHODS

Because *P. cubensis* is an obligate parasite, live-plant assays are necessary for both the pathotype determination and fungicide resistance experiments.

Pathotype determination. Lebeda and Widerlechner describe the detailed methods for pathotype determination of *P. cubensis*, as well as a set of twelve differential cucurbit hosts (Table 2) (3). In order to determine which pathotypes are present today or if a new pathotype has evolved or migrated to the U.S., these twelve cucurbit differentials were grown in the NC State Phytotron. A cork borer (13mm diam) was used to cut out leaf disks from leaves 6-8 weeks old. The leaf disks were inoculated with 3 droplets of a 10^4-zoosporaniga/mL suspension. These disks were placed in moist boxes and kept in incubators at 18°dark/22°C light (12 hr photoperiod) and inspected for infection and sporulation daily from day 3 through 8.

Fungicide resistance assays. From 2004 through 2006 over 40 isolates of *P. cubensis* were collected from diseased cucurbits in 9 states east of the Mississippi River. Cucumber plants were grown in the NC State University Phytotron growth chambers to the two-true leaves stage and sprayed with mefenoxam (0, 0.01, 0.1, 1.0, 10, 100 ppm) and azoxystrobin (0, 0.001, 0.01, 0.1, 1.0, 10, 100 ppm). After 24 hours, plants were misted with *P. cubensis* (10^4 zoosporaniga/mL) and incubated for 7 days at 18°dark/22°C light (12 hr photoperiod) and assessed for disease incidence and severity.

RESULTS

Pathotype determination. Based on the compatibility of isolates to the host differentials, specifically *Citrullus lanatus*, preliminary results (Table 2) suggest that contemporary populations of *P. cubensis* in North Carolina are different from the pathotypes described in the U.S. by Thomas et al. in 1987 (5).

Table 2. Preliminary Results of Pathotype Assay

Species	Isolate C	Isolate T	NC 2006
Cucumis sativus	+++	+++	+++
C. melo var. *reticulatus*	+++	+++	
C. melo var. *conomon*	+++	+++	
C. melo var. *acidulus*	+++	+++	
Citrullus lanatus	+++	+++	-
Cucurbita maxima	+	+++	+++
Cucurbita pepo	+	+++	+++
Cucurbita moschata	-	+++	
Benincasa hispida	+	+	+
Luffa acutangula	-	+	
Luffa cylinidrica	-	+/-	-
Mormordica charantia	+/-	+/-	
Lagenaria siceraria	nt	nt	-

U.S. isolates. Isolate C originally described on *Cucumis melo* var. *reticulatus* in South Carolina and Isolate T originally described on *Cucurbita pepo* L. in Texas in 1987 (5).
Rating. Highly compatible = +++, Compatible = +, Lesions, no sporulation = +/-, Not compatible = -

Fungicide resistance assays. Five Isolates from North Carolina, one from South Carolina and Indiana were tested and there was no reduction in disease incidence or severity at any fungicide concentration used for mefenoxam or azoxystrobin.

DISCUSSION

As *Pseudoperonospora cubensis* continues to affect the cucurbit crops east of the Mississippi River, growers are concerned about which of their crops will be affected and how to control the pathogen. Preliminary results suggest that there is a new pathotype present and that it carries resistance to mefenoxam and the strobilurin fungicides. Effective fungicide programs have been identified in field trials and a study is underway to determine the pathotype(s) of field populations.

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Pathogenicity of *Pythium* species associated with Pythium root dysfunction in North Carolina.

J.P. Kerns*, T.W. Rufty, D.M. Benson*, H.D. Shew*, and L.P. Tredway*
*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. However, it appears that the pathogen is active during the cooler temperatures of the fall and spring and the symptoms are not evident until the summer months when heat and drought stress are most severe. To shed light on Pythium root dysfunction, pathogenicity of a subsample (n=7) of our isolates (n=80) will be determined using mature creeping bentgrass plants grown in USGA specification sand. Pathogenicity of *P. volutum* and *P. torulosum*, the most frequently isolated *Pythium* species isolated from our samples, isolates will be determined. In addition, the optimal infection temperature will be determined using *P. volutum* isolates only.

Materials and Methods:

Methods to determine pathogenicity of *P. volutum* and *P. torulosum* isolates.

- Creeping bentgrass (*Agrostis stolenifera)* was seeded into conetainers containing USGA sand.
- Plants grown in greenhouse for six weeks.
- After six weeks plant roots were cut at 5cm and 5-7 infested grass blades were place on fresh sand medium.
- The inoculum was allowed to incubate for 4 weeks at 24/16°C.
- After 4 weeks at 24/16 C, the temperature was raised to 32/26 C to induce foliar symptoms.
- Treatments arranged in a CRD design with 6 replications.
- Clippings were collected every two weeks, dried, and weighed to assess foliar growth rate.
- Disease severity was visually evaluated every week for 5 weeks.
- Disease severity scale was 0=0%, 5=50%, and 10=100% disease per cone-tainer.
- Root mass and root depth measurements were taken prior to raising the temperature and 4 weeks after raising the temperature to determine the effect of the pathogen on creeping bentgrass roots.
- Experiment was repeated

Methods to Determine the Optimal Temperature for Infection by *P. volutum*

- Creeping bentgrass (*Agrostis stolenifera)* was seeded into cone-tainers containing USGA sand.
- Plants grown in greenhouse for six weeks.
- After six weeks plant roots were cut at 5cm and 5-7 infested grass blades were place on fresh sand medium.
- Four sets of inoculated cone-tainers were placed in four different chambers set at 12, 16, 20, and 24 C for 4 weeks.

- After 4 weeks, the temperature was raised in each chamber to 32/26 C to induce foliar symptoms.
- Disease severity was visually estimated on a scale of 0-10 described above.
- Root mass and root depth measurements were taken prior to raising the temperature and 4 weeks after raising the temperature to determine the effect of the pathogen on creeping bentgrass roots.
- Experiment will be replicated 3 times rotating chambers each time.
- Only one replication has been completed at this time.

Results:

P. volutum is highly aggressive to creeping bentgrass roots, whereas *P. torulosum* is not very aggressive compared to the uninoculated control (Fig. 1). Root depth and mass was not reduced prior to inducing the heat stress treatment, yet after exposure to 32/26 for 5 weeks plants inoculated with *P. volutum* exhibited severe reductions in root mass and depth (Fig. 2). Results were similar when the experiment was repeated.

It appears that the optimal infection temperature range is between 16 and 24 C. However, only one run of this experiment has been completed and the data has not been fully summarized yet.

Conclusions:

- *P. volutum* is highly aggressive towards creeping bentgrass roots.
- It appears the optimal infection temperature is between 16 and 24 C from the pathogenicity inoculations.
- *P. volutum* does not alter root depth or mass prior to inducing a heat stress; rather roots infected with *P. volutum* appear to be more sensitive to heat stress.
- Based on frequency of isolation and aggressiveness in pathogenicity experiments, *P. volutum* is the most important causal agent of Pythium root dysfunction in North Carolina.

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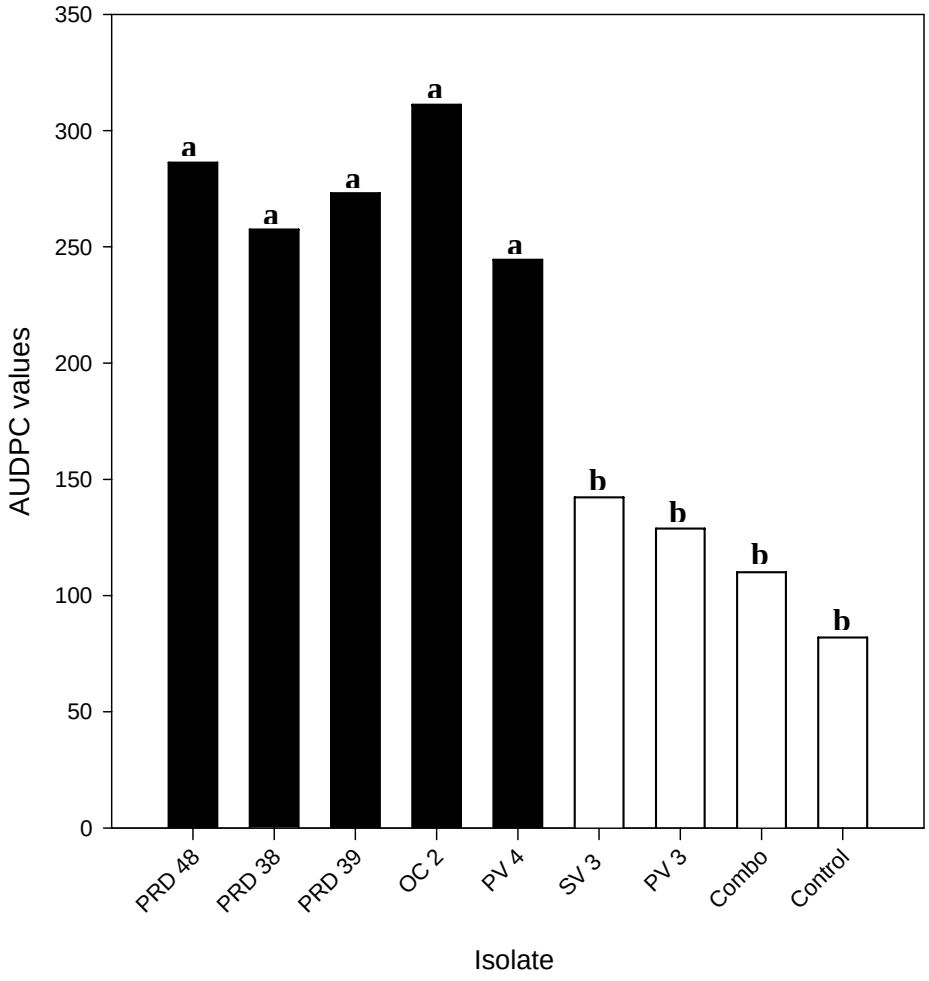


Figure 1. Area Under the Disease Progress Curve (AUDPC) values for *P. volutum* (black bars) and *P. torulosum* (SV 3 and PV 3) isolates. AUDPC values with the same letter are not statistically different (Waller-Duncan k=100).

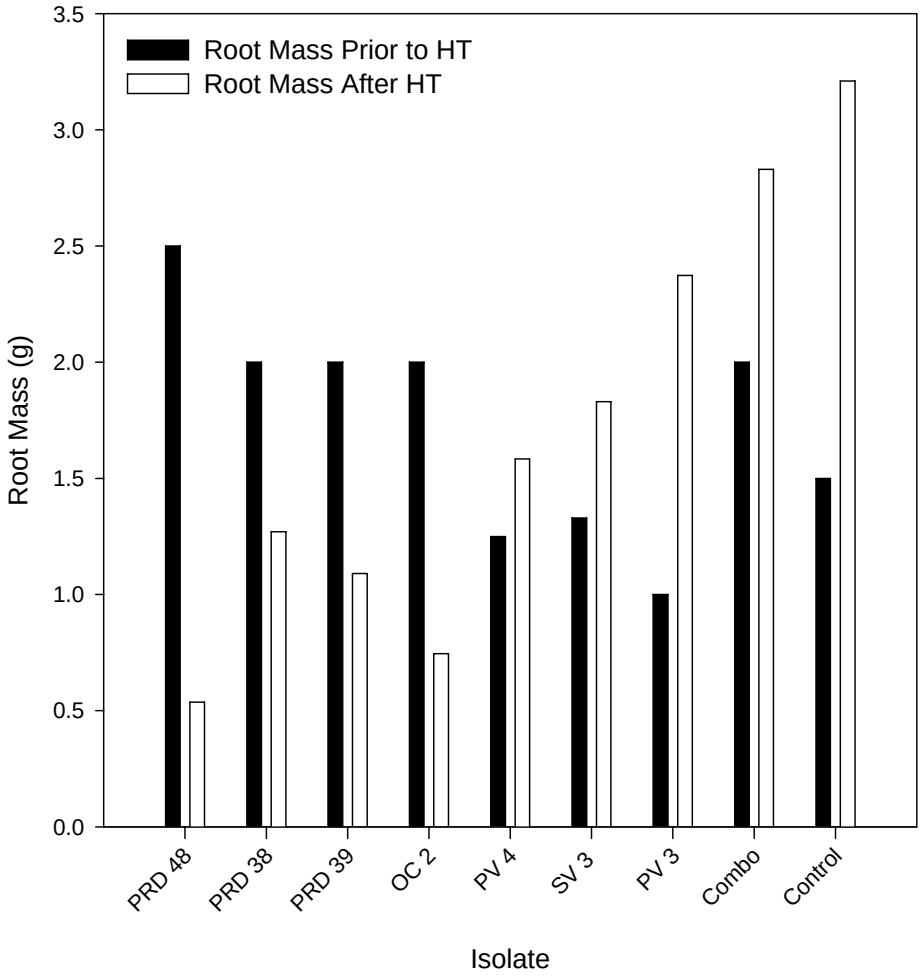


Figure 2. Root mass in grams of creeping bentgrass roots measured after 4 weeks of incubation with the pathogens at 24/16 C (black bars) and after 5 weeks at 32/26 C (white bars). All *P. volutum* isolates (PRD 48, 38, 39, OC 2, and PV 4) significantly reduced root mass and depth (not shown) after 5 weeks at 32/26 C. The *P. torulosum* (SV 3, PV 3, and Combo) isolates did not significantly reduce root mass or depth compared to the uninoculated control.

This research was presented at the Agronomy meetings in Indianapolis in 2006 by Jim Kerns. He won 1st place in the graduate student oral competition in the C-5 division. Some of the results from the inoculation experiment were published in a disease note in Plant disease, which was accepted in Jan of 2007.

Genetic Diversity Among Isolates Of *Peronospora Tabacina* From Tobacco.

Dr. Jean Beagle Ristaino and Monica Blanco
Plant Pathology

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. Blue mold spores may be transported across states, threatening tobacco production hundreds to thousands of miles away from their point of origin [1,2, 3,].

The North American Plant Disease Forecasting Center developed at NC State University has operated the Tobacco Blue Mold Warning System (TBMWS) since 1995 [3]. The TBMWS provides accurate and up-to-date warnings that assist tobacco farmers in the timing and application of appropriate control strategies for blue mold epidemics.

Peronospora tabacina is an obligate parasite and cannot be cultured *in vitro*. Sporangiospores of *Peronospora tabacina* do not produce zoospores, and infection occurs via direct germination. The asexual sporangiospores of the pathogen can be dispersed thousands of kilometers and are the primary source of inoculum for epidemics. Sporangiospores may be spread yearly from the southeastern United States on wild tobacco in Texas or may originate in Caribbean countries and move northward and infect tobacco as it is planted in fields. Yearly, epidemics reach most tobacco growing areas in the US from Florida to Canada and significant crop loss can occur. 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.

We developed a PCR primer called Ptab that can be used to specifically identify the blue mold pathogen in infected tissue. We have also developed diagnostic PCR-RFLP (restriction

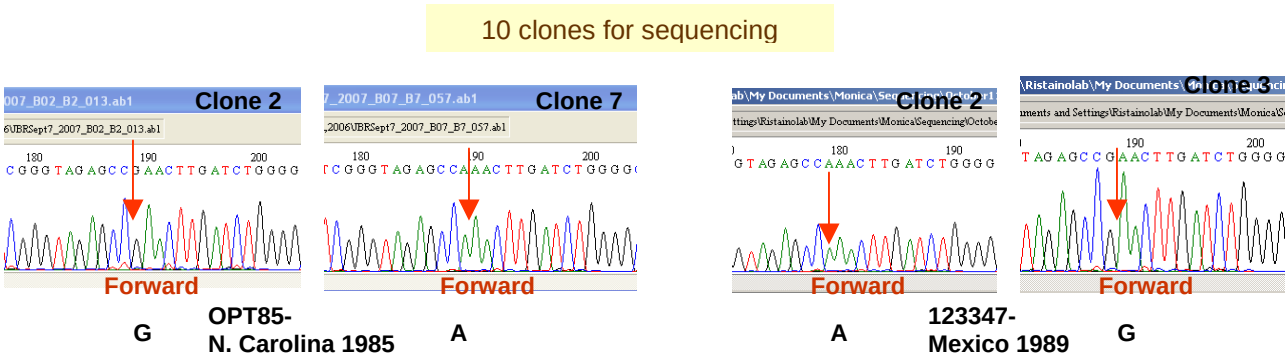
fragment length polymorphisms) fingerprints that allow us to distinguish all the major tobacco leaf pathogens (4).

We have collected tobacco blue mold isolates and transferred them to cryostorage and tobacco tissue culture. We are maintaining the largest preserved collection of tobacco blue mold isolates in the world. Kelly Ivors, Charlie Main, Tom Melton, Paul Shoemaker, Bill Nesmith and county agents from many states have deposited 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. This is no trivial task since the pathogen is an obligate and will not grow on culture media. In 2006 we added 25 isolates to the collection and it now has 229 samples. The collection includes isolates from the USA and international samples from Mexico and Dominican Republic, and Western Europe. Our lab now houses the Fort Detrick, USDA International blue mold collection. Within USA, we have samples from North Carolina, Connecticut, Florida, Georgia, Kentucky, Maryland, Pennsylvania and Texas. We obtained samples from the Dominican Republic and Nicaragua. We have been unable after repeated attempts to collect isolates from Cuba but are maintaining communications with Tobacco researchers in that country for future collections. I visited Cuba in 2004 and presented a talk on blue mold.

The objectives of the project are to: 1) Develop gene genealogies using mitochondrial and nuclear gene sequences and DNA fingerprinting to track migrations of the tobacco blue mold pathogen and study genetic structure of pathogen populations.

We are examining the genetic structure of populations of *P. tabacina* using gene sequencing of specific mitochondrial and nuclear genes. Several mitochondrial and nuclear gene regions were successfully amplified and sequenced including the P3 region (genes *rpl*5, *rpl*14 and tRNA's); P6 (part of *trn*R, *nad*4L, part of *nad*1), Mt1/6 (genes *trn*Y and r*ns*); cytochrome c oxidase subunit 2 gene (*cox*2); IgCox (intergenic region between *cox*1 and *cox*2); NADH dehydrogenase subunit 1 gene (*nad*1); Ras-related protein (*Ypt*1 gene); Beta tubulin (β-tub); some regions of the nuclear large subunit (LSU) ribosomal DNA and Igs2 region. The *cox*2,

*Igs*2, P3, *Igs*3/7, *Ypt*1 and β-tub showed variation (possible heterozygous sites) or sites that can be considered informative for phylogenetic studies. We have cloned several of these gene regions (*Igs*3/7, *Ypt*1, β-tub) from an isolate from France, NC and Mexico and have found genetically variable sites that will allow us to name haplotypes in regional populations of *P. tabacina* for the first time (Fig. 1). The results are exciting, since we will now be able to track migrations by comparing haplotype frequencies across populations.



We have found some shared haplotypes between Mexico and the US. However, further work is underway to clone and sequence additional informative sites from both nuclear and mitochondrial gene regions of more isolates and to differentiate haplotypes among regional populations.

2) Develop a real time PCR assay using the Ptab primer for blue mold diagnostics in the field.

A real time PCR assay is now under development (5). A new set of primers nested within this 764 bp region of DNA have been tested that are specific for *P. tabacina* and not other reported *Peronospora* species or tobacco pathogens. A TaqMan probe was developed that will be used with the primers to amplify an 86 bp amplicon with an ABI Prism 7300 real time PCR machine. Preliminary experiments have been conducted. This assay will be useful to diagnose the presence of this pathogen in infected tissue.

POTENTIAL SCIENTIFIC, ECONOMIC, AND OR SOCIAL IMPACTS

Blue mold control requires timely fungicide applications. The Tobacco Blue Mold Warning System tells growers when weather conditions are appropriate for disease and recommend sprays applications. However, the system has never been validated by sampling actual populations from potential sources and studying their genetic structure. It is important to understand the source of inoculum for epidemics in the US. We have been working for several years collecting and developing the appropriate genetic tools to track regional populations. We now have the appropriate tools in place and are making substantial progress. We plan to incorporate the genotypic data we generate from this research into the forecasting system. These tools combined should lead to improved predictability of the forecasting system and improved disease control for growers.

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Effect Of Temperature On Soybean Fatty Acid Composition And Pathogen Defense Gene Expression

Xue, HQ[1] and Upchurch, RG [2, 3]
[1]Crop Science Department, [2]ARS Soybean & Nitrogen Fixation Unit, [3]Plant 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 Phytotron portion of the experiment concluded with the final harvest of mature seed on January 24, 2006.

Initially, all three chambers were set for a 26/22 ^{o}C (normal) with a 12 hr day. When seed pods had developed to the R5 stage, the temperature settings of two of the chambers were changed to 22/18^{o}C (cool) or 34/26 ^{o}C (warm), while maintaining the12 hr day. Thus, seed development of the three lines continued to maturity in three different air temperature environments. Seeds were harvested at 35 days after flowering (DAF) and at maturity for experiments. Research progress for 2006 includes following:

1) Fatty acid analysis showed that all three N303 lines responded to the air temperature treatments similarly. The warm treatment produced seed containing 64 % oleic acid and 23 % linoleic acid, the normal temperature, seeds with 34 % oleic and 47 % linoleic acid, and the cool temperature, seeds with 26 % oleic and 53 % linoleic acid. The oleic/linoleic acid contents of seeds at 35 DAF and seeds at maturity were similar.

2) The susceptibility of mature seeds with significantly different oleic/linoleic content to colonization at 23 ^{o}C by the fungal pathogens *Cercospora kikuchii* and *Diaporthe phaseolorum* has been determined. The data are being statistically analyzed.

3) The expression of the defense signaling genes PR-1 and PDF1.2 in soybean seed (35 DAF) with significantly different oleic/linoleic content following induction by inoculation with the two fungal pathogens or by treatment with salicyclic and jasmonic acids has been measured at 23 ^{o}C. The data are being statistically analyzed.

PROJECTS CONDUCTED IN THE PHYTOTRON 2006

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Genetic Mechanisms Of Ozone Sensitivity In Arabidopsis And Soybean

Burkey, K. & F. Booker
Physiological And Bichemical Responses To Ozone

Morgan, P., K Burkey & F. Booker
Peroxidase Knockout Screening In Elevated Ozone

Crop Science

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Soil Ph Effects On Microbiology & Plant Function In Bermuda Grass

Burton, J.
Effect Of Day/Night Temp. Oleic Acid In Soybean

Cardinal, A
Chloroplast Fluorescence Studies Of Soybean Leaves

Coles, N & J. Holland
Verification Of Maize Photoperiod QTL

Haigler, C. & B. Singh
Investigation Of Novel Mechanisms Of Cotton Fiber Development

Haigler, C. & B. Singh
Function & Regulation Of Arabidopsis Tracheary Element Peptidase

Hixon, A., & T. Rufty
Environmental Fate Of Simazine In Bermudagrass

Holland, J., J.A. Ruiz Coral & G Vanesbroeck
Cardinal Temp. & Thermal Times For Maize & Teosinte

Holland, J.
A Vernalization Response To Maize

Lewis, R.
Evaluation Of Tobacco Resistance Gene Combinations For Their Effectiveness Against PVY Infection

Qu, R & K. Azhakanandam
Isolation Of Exon-Junction Complex (Ejc) And Study The Function Of Y14 Amd Mago Genes

Qu, R. & S. Dong
Agrobacterium-Mediated Transformation Of Tall Fescue For Disease Resistance And Drought Tolerance

Qu, R. & R. Li
St. Augustinegrass Breeding For Improved Agronomic Traits

Qu, R. & T. Tuong
Expression Of Rubi3 Promoter In Rice Plants

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Rufty, T. & G. Place
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Seth Carley, D., T. Rufty & D. Bowman
Evaluation Of Possible Growth Effects Of Pigment Application On Turf Grass Under 2 Temperature Regimes

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Burton, J.
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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