

Phytotron Report 2009



NC STATE UNIVERSITY

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Cover: Cotton spp.

NCSU Phytotron
A Controlled Environment Facility
Annual Report 2009

North Carolina State University

College of Agriculture and Life Sciences
Tomorrow's Science and Technology...Today

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Phytotron Operation: Year in Review

Carole H. Saravitz

Usage by Chamber

Usage for all growth chambers in 2009 was 107% of the recommended optimal occupancy, or 87% 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 63% optimal occupancy rate. For 2009, total A-chamber usage was 137% the recommended optimal occupancy. Usage of B-chambers was at 89% and C-chambers, 91 % for the year.

During 2009 the glasshouses had an optimal occupancy rate of 81%. A large portion of the glasshouse space allocation was utilized for studies with student projects: turfgrass (Qu, Crop Science), cotton (Haigler, Crop Science), and cucumbers (Holmes, Plant Pathology).

Two walk-in rooms equipped with high intensity discharge lamps (HID) were in use during 92% of the year for studies examining Germplasm Enhancement in Cotton Using Wild Related Species (Kuraparthi, Crop Science) And Fine-Mapping of a Photoperiod Response Gene In Maize, (Holland, Crop Science).

Usage by Department

96 different projects were conducted in the Phytotron during 2009 by faculty and students from 10 departments in the Colleges of Agriculture and Life Sciences, and Forestry (Table 2). The Crop Science Department used the largest amount of space in 2009 (nearly 38%, for 34 different projects). Secondly, the Plant Pathology Department used more than 15% of the space for 24 projects. The Plant Biology Department used more than 8% of the space for 16 projects, and Entomology used over 4% for 3 projects. Horticultural Science had a space use allocation of approximately 2%.

Usage by Crop Type

Nearly 27% of the space used in the Phytotron during 2009 was used to grow Soybeans (Table 3). Research with other agronomic crops included tobacco (5.1%), cotton (4.1%), Peanut (2.3%) and corn (2.2%). Space for research on vegetable crops used more than 6% of the space

in 2009, weeds, 4.9%, grain crops, 5.7% and for insects, 2.4%. The 'Demonstration' category (1.9%) included space for plants grown for display during tours of the facility and classroom use.

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, and crop science; as well as elementary school classes. Students from Expanding Your Horizons, St Mary's Science Camp and various science camps have also toured the Phytotron. The Association of Education and Research Greenhouse Curators members toured the Phytotron during their annual meeting held jointly between Duke University and NC State University. Visitors also came from the Belgium and China.

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. Groups larger than 10-12 people require that simultaneous tours must be provided by the Director, and Research Unit Manager. Tours must be booked in advance. 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, etc. Employment of part-time assistance for off-campus users can be arranged through the Director. Space use request forms are available on our website at <http://www.ncsu.edu/phytotron/application.html>.

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

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

Table 1. **CHAMBER USAGE SUMMARY, 2009**

Chamber		% Optimal	% Maximum
A-chambers ¹	(17 individual)	147	92
A-chambers ²	(5 standard)	101	63
A-chambers ¹	(22)	137	86
B-chambers ¹	(10)	89	89
C-chambers ¹	(22)	91	91
Glasshouses	(5)	81	65
HID Walk-in	(2)	92	92
Tall Chamber	(1)	91	91

* Dimensions of Chambers are: Utilization of all growth chambers during 2009:

A = 8' x 12' x 7'h

Optimal Usage = 107%

B = 8' x 4' x 7'h

Maximal Usage = 87%

C = 4' x 3' x 4'h

H = 10' x 6' x 8'h

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

Table 2. **DEPARTMENT USAGE SUMMARY, 2009**

Department	% Total Use-Days	# Projects
Crop Science	37.9	34
Entomology	4.1	3
Forest Resources	2.0	2
Genetics	0.5	2
Horticultural Science	1.4	4
Microbiology	0.5	2
Phytotron	2.5	
PlantBiology	8.3	16
PlantPathology	15.5	24
Soil Science	0.4	3
Visitors	26.0	3
Zoology	0.9	1
	100	96

Table 3. **CROP TYPE SUMMARY, 2009**

Crop	% Total Use-Days
Arabidopsis	6.9%
Corn	2.2%
Cotton	4.1%
Demonstration ^a	1.9%
Fruit ^b	1.0%
Grain ^c	5.7%
Grass ^d	2.4%
Insects ^e	2.4%
Maintenance	1.8%
Ornamentals ^f	1.4%
Other ^g	14.9%
Peanut	2.3%
Rice	0.5%
Soybean	26.7%
Tobacco	5.1%
Trees ^h	3.2%
Turfgrass ⁱ	6.3%
Vegetables ^j	6.1%
Weeds ^k	4.9%

Includes:

^a Corn, Himalayan Barley, Marigolds, Mung Beans, Peas, Pigweed.

^b Cantalope

^c Oats, Wheat,

^d Switchgrass, St. Augustine Grass

^e Arthropods

^f Helleborus, Roses

^g Artemisia, Annual Legume Cover Crops, Canola, Clover, Pyxie-moss (*Pyxidantha brevifolia*)

^h Dogwood, Poplar

ⁱ Bentgrass (*Agrostis palustris*), Bermudagrass, and Tall Fescue

^j Beans, Cucumber, Potato, Tomato

^k *Commelina bengalensis*, Giant Salvina, Water Hyacinth

Air Pollutant Ozone Effects on Plants – A Demonstration Laboratory Exercise with Ozone-Sensitive and -Tolerant Snap Bean Lines

Fitzgerald Booker, Kent Burkey, Erin Silva, Jeff Barton and Walt Pursley

USDA-ARS Plant Science Research Unit

Departments of Crop Science, NC State University

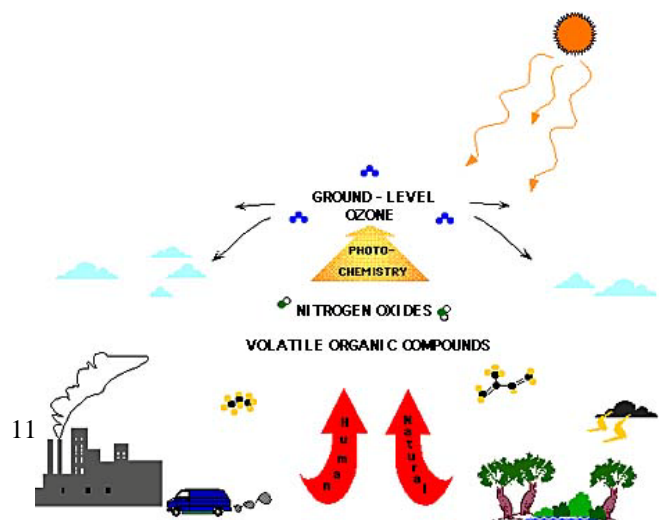


Figure 1. Effect of ambient ozone pollution on tolerant (R123, left) and sensitive (S156, right) snap bean lines (USDA-ARS Plant Science Research Unit, Raleigh, NC).

Introduction

A laboratory exercise was developed to demonstrate the physiological and growth-inhibiting effects of air pollutant ozone (O_3) on plants. Two closely-related cultivars of snap bean (*Phaseolus vulgaris* L.) with different sensitivity to O_3 were used in this exercise to highlight the genetic component of these responses (Fig. 1) (Burkey et al., 2005). The laboratory covered stomatal control of O_3 uptake by plants, visible O_3 injury, and effects on photosynthesis and biomass production. Students from ET202 (Terri Litzenberger, instructor) and CS714 (Randy Wells, instructor) learned how to measure some plant responses to O_3 and constructed a data set used for a laboratory report.

Current ground-level O_3 concentrations suppress biomass and yield in agronomically-important species in many regions worldwide. Ambient O_3 suppresses yields of susceptible crops such as soybean, cotton, peanut, rice and wheat by 5 to 15% annually (Booker et al.,



2009). Current losses to US agriculture are substantial: a 25% reduction in ambient O₃ would benefit US agriculture by \$5.2 billion annually (Adams and Horst, 2003). Improved plant tolerance to O₃ is vital to meeting future global food and biomass demands. Implementation of O₃ precursor emission controls worldwide is also important for reducing impacts on vegetation, improving human health and curtailing global warming. After CO₂ and methane, O₃ is the third most important greenhouse gas.

Methods and Materials

Plant culture and O₃ treatment

Ozone-sensitive (S156) and -tolerant (R123) snap bean lines were sown in 6" pots containing standard Phytotron potting medium and grown for 18 d in the Phytotron greenhouse at 26/22 °C day/night temperature. Plants were then transferred to four continuous-stirred tank reactors (CSTR) in a walk-in A chamber in the Phytotron. The CSTRs are Teflon-covered, cylindrical chambers that blend charcoal-filtered (virtually O₃-free) air with supplemental O₃ generated via electrical discharge through dry oxygen. Ozone treatments were administered using a computerized feedback-control system and mass-flow controllers. Snap bean lines were treated with either clean air (10 ppb O₃) or a daily average of 80 ppb O₃ for 14 days in the CSTRs. Throughout the experiment, plants received Phytotron nutrient solution or water as needed.

Stomatal conductance and photosynthesis measurements

Three hours prior to class time, two plants from each treatment combination (low O₃-S156; low O₃-R123; high O₃-S156; high O₃-R123) were placed in each of two nearby unoccupied A chambers for acclimation. Plants were well-watered at the time.

Two plants from each treatment combination in one A chamber were used for stomatal conductance measurements while two other plants from each treatment were used for photosynthesis measurements in the second A chamber. Stomatal conductance and photosynthesis measurements on upper canopy leaves were made with steady-state porometers (Li-1600M) and Li-Cor 6400 photosynthesis systems, respectively.

Instrument theory and operation was explained to the class. Teams of two students then made measurements using each instrument and recorded the data. Because of time constraints, A/C_i measurements with the Li-6400 systems were made after class on two plants from each treatment combination to determine the maximum rate of Rubisco carboxylation (V_{cmax}), the rate of electron transport (J) and daytime respiration (Rd) (Dubois et al., 2007). To construct the A/C_i curves, net photosynthesis measurements were made at six CO₂ concentrations ranging from 150 to 1550 $\mu\text{mol CO}_2$. Relative humidity was maintained at about 45% and photosynthetic photon flux was 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Foliar injury, leaf area and biomass measurements

The extent of visible injury on leaves near the base of the plant and at the top of the plant canopy was estimated using a scale of 0 – 10 (no injury to 100% discolored).

All the leaves and pods from each plant were then removed and leaf area was measured using a leaf area meter (Li-Cor Model 3100C). Leaves and stems were combined and weighed while pods were weighed separately.

Statistical analysis

Gas-exchange, visible injury and biomass data from the two class laboratories were pooled for this analysis. Results were statistically analyzed by means comparison using analysis of variance techniques (PROC MIXED, PC SAS for Windows, ver. 9.2). Data were checked for normality of distribution and homogeneity of variance prior to the analysis. Stomatal conductance data were log-transformed for the analysis. Multiple comparisons among treatment combinations were made using the Tukey-Kramer method.

Results

Visible injury, gas-exchange and A/C_i curves

Ozone-tolerant (R-123) plants showed only mild discoloration due to O₃ while the sensitive line (S-156) exhibited extensive foliar injury (Table 1). Net photosynthesis (A_n) declined by 13% in R-123 and by 60% in S-156 in the O₃ treatment. Stomatal conductance (g_s) was not significantly different between snap bean lines in the clean-air treatment, but it was 39% and 64% lower in the tolerant and sensitive plants, respectively, in the O₃ treatment. Intercellular CO₂ concentration (C_i) was not significantly different between cultivars or gas treatments.

Table 1. Effects of O₃ on visible foliar injury, net photosynthesis (A_n), stomatal conductance (g_s) and intercellular CO₂ concentration (C_i) in O₃-tolerant (R-123) and -sensitive (S-156) snap bean lines. Values are LS-MEANS \pm SE. Values followed by a different lower-case letter were significantly different between treatments within a cultivar. Values followed a different upper-case letter were significantly different between cultivars within treatments. $P \leq 0.05$.

Parameter	Treatment	Tolerant line (R-123)	Sensitive line (S-156)
Visible injury (%)	Clean-air	18 \pm 5 ^{a, A}	25 \pm 5 ^{a, A}
	O ₃	31 \pm 5 ^{a, A}	81 \pm 5 ^{b, B}
A_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Clean-air	11.1 \pm 1.2 ^{a, A}	13.8 \pm 1.2 ^{a, A}
	O ₃	9.7 \pm 1.2 ^{a, A}	5.5 \pm 1.2 ^{b, A}
g_s ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Clean-air	221 \pm 25 ^{a, A}	289 \pm 23 ^{a, A}
	O ₃	135 \pm 24 ^{b, A}	103 \pm 22 ^{b, A}
C_i ($\mu\text{mol CO}_2$)	Clean-air	296 \pm 19 ^{a, A}	306 \pm 19 ^{a, A}
	O ₃	250 \pm 19 ^{a, A}	298 \pm 18 ^{a, A}

Net photosynthesis measurements at a range of CO₂ concentrations (A/C_i curves) were used to determine several photosynthetic parameters in the two snap bean cultivars (Table 2). The maximum rate of carboxylation ($V_{c, \text{max}}$), a measurement of Rubisco activity, was similar in the two cultivars grown in clean-air and between the clean-air and O₃ treatment in R-123. However, $V_{c, \text{max}}$ in S-156 was halved by the O₃ treatment. The rates of photosynthetic electron transport (J) and daytime respiration (R_d) were not significantly different between cultivars or O₃ treatments. However, statistical power to detect differences was limited by the small data set (two plants per treatment combination).

Table 2. Effects of O₃ on the maximum rate of Rubisco carboxylation (V_{c,max}), the rate of electron transport (J) and daytime respiration (Rd) in O₃-tolerant (R-123) and -sensitive (S-156) snap bean lines. Values are LS-MEANS ± SE. Values followed by a different lower-case letter were significantly different between treatments within a cultivar. Values followed a different upper-case letter were significantly different between cultivars within treatments. *P* ≤ 0.05.

Parameter	Treatment	Tolerant line (R-123)	Sensitive line (S-156)
V _{c,max} (μmol CO ₂ m ⁻² s ⁻¹)	Clean-air	67.3 ± 16.0 ^{a, A}	64.6 ± 12.1 ^{a, A}
	O ₃	54.4 ± 12.0 ^{a, A}	29.8 ± 4.4 ^{b, B}
J	Clean-air	66.2 ± 10.2 ^{a, A}	70.9 ± 7.1 ^{a, A}
	O ₃	67.9 ± 9.1 ^{a, A}	57.8 ± 4.5 ^{a, A}
Rd (μmol CO ₂ m ⁻² s ⁻¹)	Clean-air	- 0.5 ± 2.1 ^{a, A}	- 1.2 ± 1.4 ^{a, A}
	O ₃	2.7 ± 1.9 ^{a, A}	2.2 ± 1.0 ^{a, A}

Leaf area and biomass

By the time O₃ treatments began, plants had transitioned from vegetative to reproductive growth, and few leaves were initiated during the treatment period. Consequently, O₃ effects on biomass occurred primarily on pod production and senescence processes (leaf drop). At harvest, there were no significant differences in leaf, stem and pod masses between cultivars in the clean-air treatments (Table 3). However, in the O₃ treatment, leaf and stem biomass was 18% lower in S-156 plants than in the control. Leaf and stem biomass of R-123 plants was not significantly affected by O₃. Likewise, only pod mass in the S-156 line was suppressed by the O₃ treatment. Pod mass of R-123 plants was not significantly affected by O₃. Leaf area of S-156 plants was 25-30% lower than that of R-123, but there were no significant O₃ effects.

Table 3. Effects of O₃ on leaf area, leaf and stem fresh mass, and pod fresh mass in O₃-tolerant (R-123) and -sensitive (S-156) snap bean lines. Values are LS-MEANS ± SE. Values followed by a different lower-case letter were significantly different between treatments within a cultivar. Values followed a different upper-case letter were significantly different between cultivars within treatments. $P \leq 0.05$.

Parameter	Treatment	Tolerant line (R-123)	Sensitive line (S-156)
Leaf and stem (g FW)	Clean-air	25.9 ± 1.3 ^{a, A}	28.3 ± 1.2 ^{a, A}
	O ₃	25.7 ± 1.2 ^{a, A}	23.3 ± 1.2 ^{b, A}
Pods (g FW)	Clean-air	31.4 ± 1.4 ^{a, A}	31.8 ± 1.4 ^{a, A}
	O ₃	32.0 ± 1.4 ^{a, A}	24.7 ± 1.4 ^{b, B}
Leaf area (cm ²)	Clean-air	727 ± 30 ^{a, A}	548 ± 29 ^{a, B}
	O ₃	692 ± 28 ^{a, A}	489 ± 29 ^{a, B}

Discussion

The damaging effects of O₃ and the different responses of the two cultivars were readily apparent to the students. The influence of genetic variability in plant sensitivity to O₃ was clearly demonstrated. The laboratory helped students develop a more informed understanding of O₃ pollution effects on crops and other vegetation.

Gas-exchange, visible injury and biomass effects observed in this experiment with snap bean were similar to those found in field studies with ambient O₃ (Booker et al., 2009; Burkey et al., 2005) and in outdoor controlled-environment chambers with added O₃ (Flowers et al., 2007). Overall, however, values for A_n , g_s , $V_{c, max}$, biomass and yield in this experiment were lower than those observed in plants grown outdoors. Short vegetative growth periods and lower light levels in the Phytotron experiments likely contributed to the differences. The relatively short time required for the experiment (6-7 weeks from planting to marketable-yield harvest, under short day lengths) is convenient for laboratory projects. The consistent difference in O₃ response between the two cultivars is a noteworthy feature of this experimental approach.

The basis for the differential O₃ sensitivity between the tolerant and sensitive snap bean lines is not entirely understood. One study suggests that the sensitive line has a lower concentration of

the antioxidant, ascorbic acid, in the intercellular fluid of leaves compared with the tolerant line (Burkey and Eason, 2002). Lower apoplastic ascorbic acid levels might increase the susceptibility S-156 plants to damaging reactive oxygen species generated from O₃ compared with R-123. Another factor contributing to O₃-sensitivity differences could involve stomatal conductance and O₃-uptake rates. Stomatal conductance controls to a major extent the amount of O₃ absorbed by the leaf. Conductance rates were similar between cultivars grown in clean-air and they declined in O₃-treated plants (Table 1). However, stomatal conductance, and hence O₃ uptake, was substantially reduced in the tolerant line, R-123, in the O₃ treatment although plants exhibited only mild O₃ symptoms. Ozone effects on A_n and V_{c, max}, which might lead to decreased stomatal conductance rates, were not detected in R-123 even though stomatal conductance and O₃ flux were reduced. Thus, the O₃-tolerant line might be able to restrict O₃ uptake more effectively than S-156 plants when subjected to O₃ stress.

Snap bean R-123 and S-156 seeds are available from K.O. Burkey upon request.

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Response of Sorghum and Maize to Vapor Pressure Deficit

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Drought stress is the major cause of crop yield loss in the US and world wide. It would be highly desirable to develop genetic traits in plants that allow yield increase under soil water deficits.

One such proposed trait is a restriction on crop water loss under conditions of high atmospheric demand, i.e., high atmospheric vapor pressure deficit, that result in high rates of transpiration. A restriction of water loss under high vapor pressure deficit would not only decreases overall water loss rates, but result in conserved soil water for use later in the season when drought may develop.

Previous studies have identified genetic variation for the response to high vapor pressure deficit in soybean and peanut, but major grass-type species have not been studied. These studies were undertaken to determine if variation in response to high vapor pressure deficit exists within the germplasm of sorghum and maize. For both species, more than twenty genotypes were selected for study based on indicators of good performance of these genotypes under water-deficit conditions in the field. The plants were grown in the greenhouse for about three weeks until tests of the vapor pressure deficit response could be made.

The vapor pressure deficit response was measured inside clear, 21-L commercial food containers (0.3-m tall, 0.3- m diameter). The containers were placed inside a growth chamber to maintain a constant light environment and temperature during the tests. By controlling the air flow and source of air introduced into the containers, plants were subjected to vapor pressure deficits in three ranges: 0-1.5 kPa, 1.5-2.5 kPa, and 2.5-3.5 kPa. Twelve containers were used at one time so commonly four genotypes with three replications were studied during each test.

The measurements of sorghum response to vapor pressure deficit have been completed and a marked divergence was identified among genotypes. One set of plants showed a continuous increase in transpiration rate with increasing vapor pressure deficit and the second set exhibited a breakpoint in transpiration rate with increasing vapor pressure deficit. Those exhibiting a breakpoint had a much decreased rate of transpiration rate above the breakpoint. Of the 26 sorghum lines tested, 17 lines exhibited a breakpoint. The breakpoint for these 17 lines ranged from 1.6 to 2.7 kPa and the rate of increase in transpiration above the breakpoint ranged from -
6.1 to 11.3 $\text{mg H}_2\text{O m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$. These results have identified considerable variability in a trait that could be exploited in breeding programs to enhance the drought tolerance of sorghum.

A similar experimental approach is to be used to study the vapor deficit response of maize. The experiments with maize have yet to be completed.

Screening St. Augustinegrass Germplasm for Resistance to *Pyricularia grisea*

Susana Milla-Lewis, Bangya Ma, Carolina Zuleta, Casey Reynolds, and Lane Tredway

Introduction

Gray leaf spot of St. Augustinegrass, *Stenotaphrum secundatum*, is caused by *Pyricularia grisea* and occurs throughout the southeastern United States. The disease develops on leaf blades as small, water-soaked lesions that become necrotic spots. As the disease progresses, necrotic spots coalesce, and cause partial or complete blighting of the leaf blades. Under favorable conditions, the disease develops rapidly, and entire grass swards can be killed within a few days. Cultural management practices often do not provide adequate gray leaf spot control due to the rapid development of the disease. Development of cultivars with genetic resistance to the fungus would be a potential alternative for managing the disease. Therefore, we are interested in looking for sources of gray leaf spot resistance among St. Augustinegrass germplasm. Identification and utilization of resistance genes would have great economic value as cultivars developed by the NCSU turfgrass breeding program have the potential of returning royalty dollars to the University. Besides the practical applications of screening germplasm for gray leaf spot resistance, we are also interested in generating publishable data regarding the effect of different isolates on disease response in St. Augustinegrass.

Materials and Methods

Turfgrass establishment and Maintenance:



A total of 62 St. Augustinegrass genotypes are being screened for resistance to *Pyricularia grisea*. These materials include 21 cultivars, 20 plant introductions, and 21 NCSU germplasm collection accessions (Table 1). Three stolons from each genotype were collected from the greenhouse and transplanted into 16 oz. Styrofoam cups containing calcined clay. The grass was maintained in the phytotron for three months by cutting to a height of 7 cm and fertilizing with 1.3 g N m⁻² weekly. A day before inoculation, the number of leaves per cup was counted for calculation of disease incidence.

Inoculation and incubation conditions:

Three different fungal isolates collected from North Carolina (LWS and SRS) and Georgia (1173&1345) were selected to represent different levels of virulence. The experimental design is a split plot with three replications. The main-plot factor consists of the *P. grisea* isolate, and the subplot factor of the plant genotype. The main plots are randomized within replications, and the genotypes are randomized within inoculation treatments. A day before inoculation, one cup from each genotype was placed in a plastic container. Cups were inoculated with 20ml of conidial suspension from a single isolate per inoculation treatment applied with an air-brush. Immediately after inoculation, plastic containers were covered to minimize moisture loss. The environmental conditions in the chamber for the first 24 hr after inoculation were 24°C, 100% relative humidity (RH), and no light. After that period, the chamber was set for 12-h days at 30°C, 12-h nights (no light) at 24°C, and 100% RH. Two auxiliary humidifiers were used to maintain relative humidity at 100% inside the growth chamber. At the beginning of each night

cycle, the cups will be misted with water and re-covered in order to maintain continuous and uniform humidity and leaf wetness.



Disease development:

The foliage in each cup was examined for gray leaf spot symptoms daily at 0800 h. The number of days between inoculation and symptom expression was recorded as the incubation period for each cup. The incubation period was considered lapsed when one or more gray leaf spot lesions were observed in a cup. Additionally, disease incidence and foliar blight incidence was recorded daily at 0800 h for 7 days beginning 24 hours after inoculation. Disease incidence was determined by counting the number of leaves exhibiting gray leaf spot symptoms in each cup and dividing by the average number of leaves per cup for that genotype. Mean lesion length was assessed daily for 7 days beginning 24 hr after inoculation. Mean lesion length was determined by measuring the length of 10 lesions per cup using a hand held caliper.

Data Analysis:

All statistical analyses were performed in SAS v. 9.2 (SAS Institute, Cary, NC). The parameters analyzed were incubation period, final disease incidence, and final mean lesion length. Several derived parameters such as disease progress rate, lesion expansion rate, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC) were also analyzed. AUDPC and AULEC were calculated using the formula

$\sum_{i=1}^{n-1} [(y_i + y_{i+1}) / 2] (t_{i+1} - t_i)$, where $I = 1, 2, 3, 4, 5, 6, 7$, y_i is disease incidence or mean lesion length on the i^{th} rating date, and t_i is the day of the i^{th} rating. For all parameters, an

analysis of variance (ANOVA) was performed to test the significance of main effects (genotype and isolate) and their interaction (genotype x isolate).

Preliminary Results



In the first run of the experiment significant differences among isolates were observed for all traits measured. LWS was the most virulent isolate and produced the most severe response for all traits. Conversely, 1173&1345 had the lowest means for all traits. Significant differences among genotypes were observed for all traits measured. Mercedes and PI 365031 were the genotypes with the worst and best disease responses, respectively. None of the interactions between main and subplots were significant with the exception of incubation period where there was a very significant ($p < 0.0001$) genotype*isolate effect.

LS Means for number of leaves with lesions on day 6, disease incidence on day 6, incubation period, area under the disease progress curve, mean lesion size on day 7, and area under the lesion expansion curve are listed on Table 2.

The PIs had clearly the highest levels of resistance, with PI 365031 ranking first for every parameter. FX 10 and Bitterblue, among the cultivars, had also excellent levels of resistance ranking among the top 10 for all six parameters. Conversely, Mercedes, Classic, Delmar, Jade were the most susceptible genotypes. A couple of diploid PIs, PI 212293 and PI410361 were very susceptible to the fungus ranking at the bottom for five out of the six parameters.

Continuing work

Three days after the end of the experiment, plants were sprayed with Clorothalanil at a rate of 3.2 fl oz 1000 sq ft to kill the fungus. Plants were sprayed twice ten days apart. Thirty days after the last spray, plant will be re-inoculated in order to perform a second run of the experiment.

Meanwhile, a second batch of plant materials has been transplanted into 8 oz Styrofoam cups in the phytotron's greenhouse in order to get a head start on turf establishment.

Table 1. List of St. Augustinegrass (*Stenotaphrum secundatum*) germplasm to be screened for gray leaf spot (*Pyricularia grisea*) resistance.

No.	Identity	Type	Species	Experiment
1	Classic	Cultivar	<i>S. secundatum</i>	Run I
2	Sapphire	Cultivar	<i>S. secundatum</i>	Run I
3	Floralawn	Cultivar	<i>S. secundatum</i>	Run I
4	Captiva	Cultivar	<i>S. secundatum</i>	Run I
5	Bitterblue	Cultivar	<i>S. secundatum</i>	Run I
6	Mercedes	Cultivar	<i>S. secundatum</i>	Run I
7	Floratom	Cultivar	<i>S. secundatum</i>	Run I
8	Seville	Cultivar	<i>S. secundatum</i>	Run I
9	Raleigh	Cultivar	<i>S. secundatum</i>	Run I
10	Deltashade	Cultivar	<i>S. secundatum</i>	Run I
11	Floratine	Cultivar	<i>S. secundatum</i>	Run I
12	Palmetto	Cultivar	<i>S. secundatum</i>	Run I
13	Jade	Cultivar	<i>S. secundatum</i>	Run I
14	Floraverde	Cultivar	<i>S. secundatum</i>	Run I
15	Delmar	Cultivar	<i>S. secundatum</i>	Run I
16	Amerishade	Cultivar	<i>S. secundatum</i>	Run I
17	Sunclipse	Cultivar	<i>S. secundatum</i>	Run I & II
18	FX-10	Cultivar	<i>S. secundatum</i>	Run I
19	TX Common	Cultivar	<i>S. secundatum</i>	Run I
20	Eclipse (MSA-31)	Cultivar	<i>S. secundatum</i>	Run I
21	Polaris	Cultivar	<i>S. secundatum</i>	Run I
22	PI 289729	Plant Introduction	<i>S. dimidatum</i>	Run I
23	PI 290888	Plant Introduction	<i>S. secundatum</i>	Run I
24	PI 291594	Plant Introduction	<i>S. secundatum</i>	Run I
25	PI 300129	Plant Introduction	<i>S. secundatum</i>	Run I
26	PI 300130	Plant Introduction	<i>S. secundatum</i>	Run I
27	PI 365031	Plant Introduction	<i>S. dimidatum</i>	Run I & II
28	PI 365032	Plant Introduction	<i>S. secundatum</i>	Run I
29	PI 410361	Plant Introduction	<i>S. secundatum</i>	Run I
30	PI 647925	Plant Introduction	<i>S. secundatum</i>	Run I
31	PI 410363	Plant Introduction	<i>S. secundatum</i>	Run I
32	PI 212293	Plant Introduction	<i>S. secundatum</i>	Run I
33	PI 410353	Plant Introduction	<i>S. secundatum</i>	Run II
34	PI 410355	Plant Introduction	<i>S. secundatum</i>	Run II
35	PI 410357	Plant Introduction	<i>S. secundatum</i>	Run II
36	PI 410360	Plant Introduction	<i>S. secundatum</i>	Run II
37	PI 410364	Plant Introduction	<i>S. secundatum</i>	Run II
38	PI 414079	Plant Introduction	<i>S. secundatum</i>	Run II

Table 1 (cont.). List of St. Augustinegrass (*Stenotaphrum secundatum*) germplasm to be screened for gray leaf spot (*Pyricularia grisea*) resistance.

No.	Identity	Type	Species	Experiment
39	PI 509038	Plant Introduction	<i>S. secundatum</i>	Run II
40	PI 509039	Plant Introduction	<i>S. secundatum</i>	Run II
41	PI 647924	Plant Introduction	<i>Stenotaphrum</i> sp.	Run II
42	T638	NCSU collection	<i>S. secundatum</i>	Run II
43	T644	NCSU collection	<i>S. secundatum</i>	Run II
44	T672	NCSU collection	<i>S. secundatum</i>	Run II
45	SV20	NCSU collection	<i>S. secundatum</i>	Run II
46	904AT4	NCSU collection	<i>S. secundatum</i>	Run II
47	106SVT3	NCSU collection	<i>S. secundatum</i>	Run II
48	GF2	NCSU collection	<i>S. secundatum</i>	Run II
49	1800S	NCSU collection	<i>S. secundatum</i>	Run II
50	Clem	NCSU collection	<i>S. secundatum</i>	Run II
51	Co2	NCSU collection	<i>S. secundatum</i>	Run II
52	Craig	NCSU collection	<i>S. secundatum</i>	Run II
53	Jones	NCSU collection	<i>S. secundatum</i>	Run II
54	Ray	NCSU collection	<i>S. secundatum</i>	Run II
55	Rebok	NCSU collection	<i>S. secundatum</i>	Run II
56	200Elm4	NCSU collection	<i>S. secundatum</i>	Run II
57	C1	NCSU collection	<i>S. secundatum</i>	Run II
58	C2	NCSU collection	<i>S. secundatum</i>	Run II
59	C3	NCSU collection	<i>S. secundatum</i>	Run II
60	C4	NCSU collection	<i>S. secundatum</i>	Run II
61	C6	NCSU collection	<i>S. secundatum</i>	Run II
62	C7	NCSU collection	<i>S. secundatum</i>	Run II

Table 2. LS Means for six parameters of gray leaf spot (*Pyricularia grisea*) response measured in a set of 32 cultivars and plant introductions of St. Augustinegrass.

Genotyp													BES T	WORST							
	LLD6		InD6		IncP		audpc		LSD7		aulec		ALL	LLD 6	InD 6	IncP <2.2	audp c	LSD 7	aule c	su m	
	LSM	Ran k	LSM	Ran k	LS M	Ran k	LSM	Ran k	LSM	Ran k	LSM	Ran k	<10	>20	>20	<2	>20	>20	>20	>20	
Amerisha	31.8	9	21	30.48	8	2.11	10	62.04	16	2.95	11	5.88	14		1		1				2
Bitterblu	15.5	6	7	25.49	7	2.11	10	54.28	11	2.81	9	5.64	10	***			1				1
Captiva	30.2	8	18	33.36	12	2.89	2	38.48	4	2.49	8	5.24	8								0
Classic	33.0	0	24	47.96	30	2.67	4	68.67	21	4.09	24	6.75	26		1	1		1	1	1	5
Delmar	37.1	1	27	42.92	22	2.22	8	64.76	20	4.34	28	6.79	27		1	1	1		1	1	5
Deltash	30.6	7	19	61.79	31	2.56	5	79.59	25	4.34	27	6.74	25			1		1	1	1	4
Eclipse	27.2	2	13	37.43	17	2.67	4	54.70	12	3.33	17	5.81	12								0
Floralwn	17.7	8	8	31.04	10	2.22	8	62.55	17	3.48	19	6.50	21				1			1	2
Floratam	21.5	6	10	39.76	20	2.11	10	80.42	26	3.51	20	6.43	20				1	1			2
Floratine	30.8	9	20	35.43	15	2.11	10	69.25	22	2.98	12	5.31	9				1	1			2
Floraverd	27.4	4	15	31.70	11	2.11	10	57.46	14	3.83	22	6.71	24				1		1	1	3
FX-10	10.1	1	3	19.07	5	2.11	10	44.04	6	1.90	6	4.51	6	***			1				1
Jade	34.2	2	25	44.19	25	2.44	6	69.77	23	4.73	30	6.94	28		1	1		1	1	1	5
Merced	38.3	3	28	46.37	27	2.00	11	105.25	30	4.22	26	7.91	31		1	1	1	1	1	1	6
Palmetto	28.6	7	16	38.82	19	2.78	3	48.39	10	3.21	14	5.85	13								0
PI21229	38.7	8	29	36.46	16	2.00	11	72.90	24	4.11	25	7.13	29		1		1	1	1	1	5
PI28972	10.2	9	4	16.10	3	2.11	10	48.05	8	0.37	2	1.15	2	***			1				1
PI29088	19.3	8	9	34.67	14	2.00	11	87.06	28	1.94	7	4.91	7				1	1			2
PI29159	13.1	6	6	20.60	6	2.33	7	43.73	5	1.24	5	3.64	5	***			1				1

4	1																			
PI300129	7.67	2	11.02	2	2.56	5	23.21	2	1.16	4	3.00	3	***						0	
PI300130	11.00	5	17.15	4	2.11	10	32.85	3	0.94	3	3.01	4	***			1			1	
PI365031	4.11	1	9.28	1	3.44	1	22.05	1	0.14	1	0.60	1	***						0	
PI410361	50.78	31	46.52	28	2.00	11	109.31	31	3.05	13	6.52	22		1	1	1	1	1	5	
PI410363	36.28	26	42.38	21	2.17	9	57.24	13	3.22	15	6.23	17		1	1	1			3	
PI647925	26.33	12	30.99	9	2.11	10	48.36	9	3.22	16	5.68	11				1			1	
Polaris	41.00	30	46.72	29	2.67	4	63.40	18	4.36	29	6.53	23		1	1			1	1	4
Raleigh	23.78	11	38.74	18	2.44	6	58.81	15	3.37	18	5.94	15							0	
Sapphire	32.89	23	43.80	23	2.00	11	97.36	29	2.92	10	5.97	16		1	1	1	1		4	
Seville	30.22	17	45.30	26	2.67	4	81.69	27	3.61	21	6.38	19			1		1	1	3	
Sunclips	32.11	22	33.38	13	2.22	8	47.06	7	4.95	31	7.23	30		1		1		1	1	4
TX Com	27.33	14	44.09	24	2.78	3	63.86	19	4.06	23	6.35	18			1			1	2	

LLD6 - number of leaves with lesions on day 6.

InD6 - disease incidence on day 6.

IncP - incubation period.

audpc - area under the disease progress curve.

LSD7 - mean lesion size on day 7.

aulec - area under the lesion expansion curve.

Screening Peanut Germplasm for Tomato Spotted Wilt Virus (TSWV) Resistance

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Introduction

Tomato spotted wilt virus (TSWV) is the causal agent of spotted wilt of peanuts. The disease has been progressively increasing in severity in the Virginia-Carolina peanut growing area since the mid-1990s. Currently, spotted wilt is one of the major diseases in the area and can dramatically reduce seed weight and yield. Although TSWV is vectored by thrips, chemical control of the thrips usually does not result in reduction of spotted wilt incidence. There are few effective cultural and chemical practices for management of the disease. Although several factors have been shown to provide some suppression, no single measure by itself has been effective under heavy disease pressure. Cultivar selection is the most important component for reducing the risk of spotted wilt. Therefore, breeding for resistant cultivars appears to have the most potential for minimizing the risk of losses to spotted wilt. Several cultivars with field resistance to TSWV have been released; however, none possesses true resistance to the virus *per se*. The identification of new sources of resistance to TSWV is an important goal of the peanut breeding program at NCSU.

When dealing with TSWV artificial inoculations in the greenhouse there are two main constraints to deal with. First, given the fact that TSWV carries its own enzymes for replication within the host, the virus is extremely sensitive to high temperatures. We require an environment which will allow accurate maintenance of temperature within $\pm 2^{\circ}\text{C}$. Secondly, plants need to be maintained in complete darkness for before and after inoculation to soften the cuticle and facilitate entry of the virus into the leaf tissue. This is hard to achieve in the greenhouse, especially without raising the temperature. Performing artificial inoculations in a controlled environment has two-fold benefits: we are able to control the temperature accurately, and to easily keep the plants in complete darkness.

The objective of this study is to evaluate peanut germplasm with different isolates of TSWV under controlled environmental conditions.

Materials and Methods

Plant Materials: Two different sets of peanut germplasm are currently being screened for TSWV resistance: (1) The core of the core of the peanut germplasm collection, which consists of 108 accessions, and (2) a set of 50 breeding lines that show moderate levels of field resistance to spotted wilt. Additionally, cultivars NC 7 and NC 9 are being used as the susceptible checks. Accession GKP 10602 (PI 276235) of the diploid ($2n-2x=20$) wild species *A. diogeni* Hoehne has been shown to be highly resistant to different isolates of TSWV and therefore is being used as the resistant check. Eight seeds from each genotype are treated with Vitavax, rolled in paper towels and placed in water to germinate. After a week, seedlings are transplanted to 4"x4" pots containing MetroMix2000 and kept at 25 C using a 14 hr light/10 hr dark photoperiod.



Artificial Inoculations: A week after transplanting, plants are inoculated with the virus. A day before and after inoculation, chamber lights are turned off to keep plants in complete darkness because etiolation softens the cuticle, facilitating entry of the virus into the plant tissue. Virus isolates SUF23 and LEW6 used in this study were obtained from Dr. Amanda Kaye of NCSU's Dept. of Plant Pathology. The isolates were collected in 2007 from naturally infected peanut plants at Suffolk, VA and Lewiston, NC. *Nicotiana benthamiana* Domin. is used as a susceptible host to increase the virus load prior to plant inoculations. All inoculations are carried out in ice-cold Tris buffer (0.01M Tris with 0.01M Na₂SO₃ and 0.01% cysteine HCl added immediately before use). Grinding materials and buffer are kept on ice before and during inoculation. Approximately 3-5 g of symptomatic tissue is collected from infected *N. benthamiana* plants and ground in inoculation buffer with a mortar and pestle. After grinding the tissue, silicon carbide (carborundrum, 600-800 mesh) is added to the suspension to facilitate wounding in order to

provide an entryway for the virus into the plant tissue. The inoculum is then rubbed on the upper and lower leaf surfaces of two leaves from each plant with a cotton swab. Plants are subsequently rinsed with water. Plants of cultivars NC 7 and NC 9 and plants of *A. diogoii* are included in each set of inoculations as the susceptible and resistant checks, respectively. An additional set of plants of cultivar NC 7 is mock inoculated (rubbed with inoculation buffer without inoculum) in each round of inoculations to serve as the negative control.



Inoculated plants are monitored for 2-3 weeks for symptom development. Final disease reactions (systemic infection = 1 or no systemic infection = 0) are recorded approximately 21 days post-inoculation. At this point, leaf samples from each inoculated plant are taken in order to conduct ELISA tests to confirm virus infection.

Experimental Design: In 2008, the experimental design used was a split plot with three replications. The main-plot factor was the virus inoculation treatment (isolate), and the subplot factor was the plant genotype. The main plots were randomized within replications, and the genotypes were randomized within inoculation treatments. An experimental unit consisted of one individual plant. Because of the variability in infection success, the number of plants per experimental unit was increased from one to five in experiments in 2009., and experimental design was changed to a randomized complete block (RCB) design with three replications where genotypes were the treatments. Only the most virulent and consistent TSWV isolate, LEW6, was used in the experiments conducted in 2009.

Results

Split plot experiments conducted in 2008: The first three runs of the experiment served to fine tune our inoculation technique. During this time we learned 1) optimum times for seed transplantation and seedling inoculation, 2) optimum times for keeping plants in darkness before and after inoculation, and 3) best virus isolates to use in terms of virulence. While fine tuning the technique, we managed to evaluate four cultivars, 56 core collection accessions and 49 breeding lines.



When doing pair wise comparisons of levels of resistance between the most interesting groups, we found that there were significant differences between cultivars and wild species, and between wild species and breeding lines. However, there were no other materials that were as resistant to this isolate as the resistant check.

RCB experiments conducted in 2009: Results were consistent across three runs of the experiment. High levels of disease incidence were observed for the susceptible checks, cultivars NC 7 and NC 9. On the contrary, no symptoms were observed for neither the resistant check, GKP 10602, nor the mock inoculation control. Significant differences in TSWV response were observed among genotypes for both runs of the experiment (Table 1). Species-derived lines of particular interest include SPT 011, SPT 108, SPT 020, SPT 028, and SPT 034.

Table 1. Adjusted mean proportion of systemically symptomatic plants and plants testing positive for TSWV infection by ELISA.

Group / by ELISA line	Systemically symptomatic plants			
	Proportion (x)	Transformed (\sqrt{x})	Proportion	Transformed (\sqrt{x})
Negative control	0.000±0.098^a	0.000±0.094^a	0.000±0.097^a	0.000±0.093^a
NC 7 negative control	0.000±0.098 ^a	0.000±0.094 ^a	0.000±0.097 ^a	0.000±0.093 ^a
<i>A. diogeni</i>	0.000±0.098^a	0.000±0.094^a	0.000±0.097^a	0.000±0.093^a
GKP 10602	0.000±0.098 ^a	0.000±0.094 ^a	0.000±0.097 ^a	0.000±0.093 ^a
<i>A. hypogaea</i> checks	0.757±0.069^β	0.858±0.066^β	0.761±0.069^γ	0.862±0.066^γ
NC 7	0.771±0.098 ^{g-k}	0.868±0.094 ^{f-i}	0.743±0.097 ^{d-h}	0.850±0.093 ^{g-hi}
NC 9	0.743±0.098 ^{g-k}	0.849±0.094 ^{e-i}	0.779±0.097 ^{d-h}	0.875±0.093 ^{g-hi}
Species-derived lines	0.630±0.020^β	0.729±0.019^β	0.599±0.020^β	0.705±0.019^β
SPT 001	0.667±0.098 ^{e-j}	0.737±0.094 ^{d-i}	0.571±0.097 ^{b-f}	0.676±0.093 ^{b-h}
SPT 002	0.844±0.154 ^{g-k}	0.888±0.147 ^{e-i}	0.841±0.152 ^{d-h}	0.879±0.146 ^{f-i}
SPT 003	0.479±0.098 ^{b-g}	0.569±0.094 ^{b-f}	0.386±0.097 ^{bc}	0.509±0.093 ^{b-e}
SPT 004	0.694±0.154 ^{d-k}	0.804±0.147 ^{c-i}	0.757±0.152 ^{c-h}	0.834±0.146 ^{d-i}
SPT 005	0.561±0.154 ^{b-j}	0.581±0.147 ^{b-h}	0.491±0.152 ^{b-e}	0.536±0.146 ^{b-g}
SPT 006	0.700±0.154 ^{d-k}	0.800±0.147 ^{c-i}	0.780±0.152 ^{d-h}	0.843±0.146 ^{e-i}
SPT 007	0.444±0.154 ^{b-g}	0.515±0.147 ^{b-e}	0.507±0.152 ^{b-f}	0.541±0.146 ^{b-g}
SPT 008	0.588±0.154 ^{c-j}	0.720±0.147 ^{c-i}	0.585±0.152 ^{b-g}	0.711±0.146 ^{b-i}
SPT 009	0.455±0.132 ^{b-g}	0.567±0.126 ^{b-f}	0.453±0.130 ^{bcd}	0.584±0.125 ^{b-h}
SPT 010	0.977±0.154 ^{jk}	0.959±0.147 ^{ghi}	0.974±0.152 ^{gh}	0.949±0.146 ^{hi}
SPT 011	0.336±0.098 ^{bcd}	0.470±0.094 ^{bc}	0.307±0.097 ^b	0.454±0.093 ^{bc}
SPT 012	0.644±0.154 ^{c-j}	0.766±0.147 ^{c-i}	0.641±0.152 ^{b-g}	0.756±0.146 ^{b-i}
SPT 013	0.977±0.154 ^{jk}	0.959±0.147 ^{ghi}	0.891±0.152 ^{e-h}	0.904±0.146 ^{f-i}
SPT 014	0.844±0.154 ^{g-k}	0.884±0.147 ^{e-i}	0.841±0.152 ^{d-h}	0.874±0.146 ^{f-i}
SPT 015	0.894±0.154 ^{h-k}	0.914±0.147 ^{e-i}	0.824±0.152 ^{d-h}	0.869±0.146 ^{f-i}
SPT 016	0.527±0.154 ^{b-i}	0.670±0.147 ^{b-h}	0.441±0.152 ^{bcd}	0.615±0.146 ^{b-h}
SPT 017	0.533±0.154 ^{b-i}	0.702±0.147 ^{c-i}	0.446±0.152 ^{bcd}	0.624±0.146 ^{b-h}
SPT 018	0.321±0.098 ^{bc}	0.514±0.094 ^{bcd}	0.321±0.097 ^b	0.473±0.093 ^{bcd}
SPT 019	0.911±0.154 ^{ijk}	0.924±0.147 ^{f-i}	0.907±0.152 ^{gh}	0.914±0.146 ^{f-i}
SPT 020	0.221±0.098 ^{ab}	0.347±0.094 ^b	0.321±0.097 ^b	0.474±0.093 ^{bcd}
SPT 021	0.677±0.106 ^{e-j}	0.795±0.102 ^{e-i}	0.502±0.105 ^{b-e}	0.654±0.101 ^{b-h}
SPT 022	0.761±0.154 ^{e-k}	0.844±0.147 ^{d-i}	0.757±0.152 ^{c-h}	0.834±0.146 ^{d-i}
SPT 023	0.977±0.154 ^{jk}	0.959±0.147 ^{ghi}	0.907±0.152 ^{gh}	0.914±0.146 ^{f-i}
SPT 024	0.492±0.132 ^{b-h}	0.707±0.127 ^{c-i}	0.524±0.131 ^{b-f}	0.738±0.125 ^{c-i}
SPT 025	0.555±0.132 ^{c-i}	0.656±0.127 ^{b-h}	0.444±0.131 ^{bcd}	0.583±0.125 ^{b-h}
SPT 026	0.467±0.132 ^{b-g}	0.663±0.127 ^{c-h}	0.469±0.131 ^{bcd}	0.670±0.125 ^{b-h}
SPT 027	0.867±0.132 ^{ijk}	0.948±0.127 ^{hi}	0.569±0.131 ^{b-f}	0.675±0.125 ^{b-h}
SPT 028	0.330±0.132 ^{bcd}	0.424±0.127 ^{bc}	0.332±0.131 ^b	0.431±0.125 ^{bc}
SPT 030	1.084±0.153 ^k	1.088±0.146 ⁱ	1.090±0.151 ^h	1.106±0.145 ⁱ
SPT 031	0.617±0.132 ^{c-j}	0.698±0.127 ^{c-h}	0.619±0.131 ^{b-g}	0.705±0.125 ^{b-h}
SPT 032	0.780±0.132 ^{e-k}	0.880±0.127 ^{e-i}	0.832±0.131 ^{d-h}	0.913±0.125 ^{g-hi}
SPT 034	0.330±0.132 ^{bcd}	0.424±0.127 ^{bc}	0.269±0.131 ^{ab}	0.380±0.125 ^b
SPT 035	0.644±0.154 ^{c-j}	0.739±0.147 ^{c-i}	0.641±0.152 ^{b-g}	0.730±0.146 ^{b-i}
SPT 036	0.577±0.154 ^{b-j}	0.733±0.147 ^{c-i}	0.441±0.152 ^{bcd}	0.629±0.146 ^{b-h}
SPT 037	0.786±0.098 ^{g-k}	0.873±0.094 ^{f-i}	0.721±0.097 ^{d-h}	0.824±0.093 ^{f-i}
SPT 038	0.567±0.132 ^{c-j}	0.643±0.127 ^{b-h}	0.369±0.131 ^{bc}	0.455±0.125 ^{bcd}
SPT 039	0.767±0.132 ^{e-k}	0.883±0.127 ^{e-i}	0.719±0.131 ^{c-h}	0.844±0.125 ^{f-i}
SPT 040	0.417±0.132 ^{b-e}	0.653±0.127 ^{b-h}	0.469±0.131 ^{bcd}	0.695±0.125 ^{b-h}
SPT 041	0.534±0.132 ^{b-i}	0.643±0.127 ^{b-h}	0.503±0.131 ^{b-e}	0.626±0.125 ^{b-h}
SPT 042	0.417±0.132 ^{b-f}	0.571±0.127 ^{b-g}	0.369±0.131 ^{bc}	0.532±0.125 ^{b-f}
SPT 043	0.567±0.132 ^{c-j}	0.668±0.127 ^{c-h}	0.569±0.131 ^{b-f}	0.675±0.125 ^{b-h}
SPT 044	0.534±0.132 ^{b-i}	0.709±0.127 ^{c-i}	0.503±0.131 ^{b-e}	0.702±0.125 ^{b-h}
SPT 045	0.480±0.132 ^{b-g}	0.600±0.127 ^{b-h}	0.482±0.131 ^{bcd}	0.607±0.125 ^{b-h}
SPT 046	0.746±0.132 ^{e-k}	0.878±0.127 ^{e-i}	0.749±0.131 ^{d-h}	0.886±0.125 ^{g-hi}
SPT 048	0.755±0.132 ^{e-k}	0.859±0.127 ^{e-i}	0.807±0.131 ^{d-h}	0.913±0.125 ^{g-hi}

α,β,γ Group means followed by the same Greek letter are not different by t-test (P<0.05).

a,b,c Line means followed by the same Roman letter are not different by t-test (P<0.05).

IMPACT:

Spotted wilt of peanuts (*A. hypogaea* L.) caused by the tomato spotted wilt tospovirus (TSWV) is currently one of the major limiting factors to peanut production in the U.S. Cultivar selection remains the most important component for reducing the risk of spotted wilt. Therefore, breeding for resistant cultivars appears to have the most potential for combating spotted wilt. Accession GKP 10602 of *Arachis diogenii* Hoehne, a diploid wild relative of peanut, is known to possess high levels of resistance to different isolates of TSWV. Resistance present in 10602 can ultimately be used for incorporation of resistance genes into improved peanut cultivars. In order to localize genetic factors controlling TSWV resistance in 10602, a genetic linkage map was constructed using an F₂ population derived from the cross *A. kuhlmannii* accession 7639 (a wild relative of peanut susceptible to TSWV) by *A. diogenii* accession 10602. Fifteen markers were found to be closely associated with TSWV resistance. We are currently working on validating these markers within F_{2,3} families from the original *A. diogenii* x *A. kuhlmannii* cross so that they might be used to aid in transferring the resistance present in *A. diogenii* GKP 10602 into elite peanut breeding materials. While work with the wild species and their derivatives provides an invaluable tool for identifying sources of resistance to all major peanut diseases, in terms of practical breeding it can be long term due to fertility issues that arise when transferring genes from the diploid species to cultivated peanut. Therefore, we are working on searching for new sources of resistance to TSWV among cultivated materials. A total of 158 genotypes including the core of the core of the peanut collection and a set of breeding lines that have shown good levels of resistance to TSWV in the field are currently being screened under controlled environmental conditions in the NCSU Phytotron.

Screen Soybean for Transpiration Sensitivity to AgNO₃

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Aquaporins have been identified in both animal and plant systems as key in regulating the flow of water into and out of cells. Recently, it was discovered that there was a restricting hydraulic conductance in the leaves of soybean PI 416937, which is a “slow wilting” genotype under drought in the field, and this restriction was hypothesized to result from low aquaporin activity. In recent studies, it was found water flow in PI 416937 was not sensitive to treatment with silver nitrate in contrast to a high sensitivity in most soybean lines. These results were interpreted as indicating the lack of an aquaporin population in PI 416937 that is sensitive to inhibition by the silver nitrate treatment. The practical benefit of the missing aquaporin population is that water flow in PI 416937 would be limited so that transpiration rates would be limited under high vapor pressure deficit.

This study was undertaken to fully characterize response to silver nitrate among the recombinant inbred line population of the cross of PI 416937 x Benning. While PI 416937 is insensitive to the silver nitrate treatment, Benning is highly sensitive to exposure to silver nitrate treatment such that there is a marked decrease in transpiration upon exposure to silver nitrate. There are approximately 150 lines in this population to be characterized.

The plants are grown in pots in the greenhouse before doing the silver nitrate tests. Once about three trifoliolate leaves have expanded, the plants are subjected to the silver nitrate tests. Four replicate plants were tested for each genotype. Late in the afternoon prior to the test, the shoots are cut from the roots and placed in flasks with water overnight in a the dark. The next morning the plants in the flask are moved to a growth chamber to be subjected to a constant light and temperature environment. After acclimating for about one hour and then measuring transpiration for another hour, the plant shoots are transferred to flasks containing 200 μ M silver nitrate. After allowing about three hours for a new stable

transpiration rate to be established, transpiration rate was measured again for one hour. The response to the silver nitrate treatment was calculated as the ratio between transpiration rate with the silver nitrate treatment vs. the initial transpiration on water.

The experiment was limited since greenhouse space to grow only 20 genotypes at one time was available. Therefore, a number of cycles of the study are required to test all 150 lines. At this time, not all genotypes have been tested. However, at this point it is clear that these tests confirmed the difference in sensitivity of PI 416937 and Benning in response to the silver nitrate treatment. The response among the progeny lines ranged from being similar to one of the parents to a level of sensitivity intermediate between the two parents. Once all progeny lines are characterized, these results will be used to identify possible correlations between the sensitivity to the silver nitrate treatment and DNA markers.

After completing the tests of the recombinant inbred population, the technique will be applied to a diversity of soybean germplasm identified to have unique slow-wilting characteristics in the field.

Turfgrass Biotechnology Program

Ron Qu
Crop Science

The well-managed NCSU Phytotron is an invaluable facility for the experiments conducted in my lab. Almost all my projects have to depend on the Phytotron to grow plants. We appreciate very much the strong support and kind assistance we received in the past year from the Phytotron staff, particularly Drs. Carole Saravitz and Janet Shurtleff. The following are the projects in my research program that used Phytotron facility in 2009:

1. In a new research project, we grew over a hundred of tissue culture regenerated or transgenic plants of perennial ryegrass at the Phytotron greenhouse.
2. Graduate student Bingwu Wang uses the Phytotron facility for hydroponical culture of tobacco plants in his project studying nicotine biosynthesis.
3. Dr. Ruyi Li used the greenhouse to grow her transgenic switchgrass plants in her project to reduce lignin in the biofuel species.
4. In a collaborative project, Drs. Dan Bowman and I conducted investigation on transgenic tall fescue plants for drought tolerance.
5. In a collaborative project, Dr. Niki Robertson and I grew rice plants in phytotron to provide immature embryos for rice transformation to evaluate drought tolerance effects of a transgene.
6. In addition, I grew transgenic Arabidopsis plants to evaluate two genes that may be critical to the intron-mediated enhancement of gene expression in plants.

2009 publications from Qu's lab resulted from use of phytotron facility

Feng C.-M., R. Qu, L. Zhou, D. Xie, and Q.-Y. Xiang (2009) Shoot regeneration of dwarf dogwood (*Cornus canadensis* L.) and morphological characterization of the regenerated plants. *Plant Cell Tiss. Org. Cult.* 97:27-37

Evolution of Resistance-Breaking in *Tomato Spotted Wilt Virus*: Response to Selection by *sw-5* Mediated Resistant Tomato.

Jessica L. Houle, James W. Moyer, & George G. Kennedy

Abstract:

The rapid evolution of resistance-breaking (RB) in RNA plant viruses circumvents human efforts to develop disease-resistant crops. Increasing selection for RB through repeated use of crops expressing the same resistance gene has important implications for their long-term utility. Understanding fitness as well as virulence of a virus under intense selection will aid in development of models to predict the consequences of using plants with dominant single gene resistance. To explore the evolution of virus fitness and virulence in RB isolates, we selected *tomato spotted wilt tospovirus* (TSWV) and cultivated tomato (*Solanum lycopersicum*) as a model system. We are testing the hypothesis that selection for RB viral phenotypes through sequential passage in hosts with the *sw-5* dominant single gene for resistance will increase virus fitness in subsequent resistant hosts and decrease virus fitness in susceptible hosts. We are also predicting that RB isolates serially transferred through susceptible hosts will be less fit when returned to a resistant host. By increasing the number of transfers in one host type, we expect to see a more significant decrease in virus fitness when returned to the alternate host type. Measurements of the variation in virulence from one host transfer to the next will be used to predict the consequences of RB epidemics in sequential cropping systems vs. systems with host alternation. Preliminary results suggest a change in viral phenotypes under selection by resistant hosts. Future directions will explore changes at the molecular level to determine the strength of selection by the host plant and make predictions about the evolution of RB in TSWV.

Introduction

Tomato spotted wilt virus (TSWV) (*Tospovirus*, *Bunyaviridae*) is a thrips-vectored plant pathogen that devastates crops and ornamentals worldwide ¹. While no single control method is effective at preventing disease spread, many scientists consider that naturally resistant varieties are a promising control option ². Unfortunately, resistance-breaking (RB) strains of TSWV have developed in resistant tomato and pepper varieties expressing

the *sw-5* and *tsw* resistance genes, respectively, rendering resistant hosts ineffective in some areas^{3 4}.



Figure 1 Tomato fruit infected with *tomato spotted wilt*

TSWV is a membrane-bound, negative strand RNA virus with three genome segments (L, M, S). Five viral genes have been linked to roles in receptor binding, replication, post-translational RNA silencing suppression, and movement in hosts⁵. By suppressing RNA silencing in plants, mutations of the S RNA encoding the nonstructural protein (NSs) are responsible for the breakdown of resistance in peppers^{6 7}. In tomatoes, the genetic determinant for loss of resistance is found on the M RNA, which encodes glycoproteins, G_n and G_c , and another nonstructural protein (NSm), which is responsible for cell-to-cell movement^{8 9}.

RB isolates of TSWV are heterogeneous mixtures of both RB and susceptible variants¹⁰. Some research suggests that viruses displaying novel characteristics like RB suffer a loss in fitness¹¹. This warrants investigation into the durability of RB strains of TSWV.

In our study, we want to determine if there is a fitness cost associated with RB in TSWV. Selection will be placed on RB isolates by passage through different combinations of

susceptible and resistant hosts to measure effects on replication and transmissibility in tomato containing the *sw-5* gene (see Fig. 2).

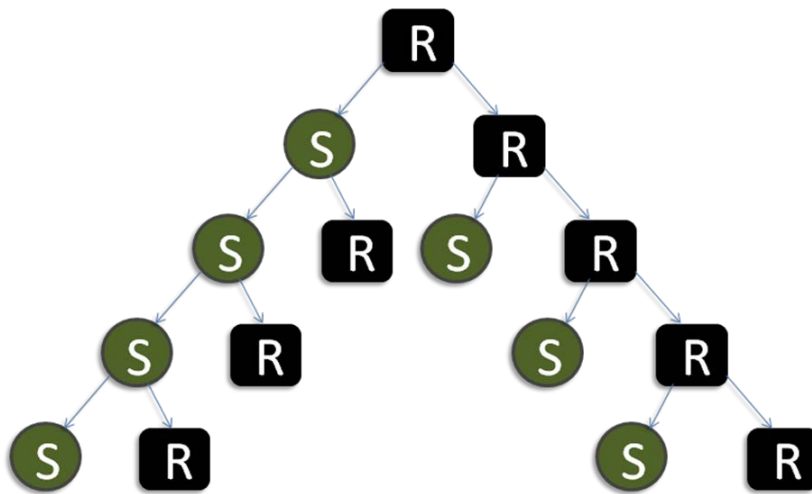


Figure 2. Path of the TSWV isolate as it moves through resistant (R) and susceptible (S) hosts in different configurations. Arrows show direction from source plant host to recipient plant host.

Methods:

Two isogenic varieties of *Solanum lycopersicum* were selected: Mountain Glory and Mountain Spring. Mountain Glory contains the *sw-5* gene for resistance to TSWV. Each group of susceptible or resistant plants contained 8 individuals that were inoculated with the virus and four control plants that were inoculated with buffer only. Seeds were planted in trays with 50:50 (sand:peat) mix and germinated in the misting chamber for 4 days before being moved to the greenhouse. For 16 days, seedlings grew at temperatures within 22-26°C and individuals with 2-4 true leaves were transplanted into 4 ½” plastic pots. At 21 days, the plants were moved to a growth chamber and placed in complete darkness at a temperature cycle of 22/26°C (12h:12h).

At 22 days, the plants were mechanically inoculated with TSWV and maintained in the dark for an additional 24 hours. For the remainder of the experiment, the plants stayed in a light:dark cycle of 12h:12h and temperature cycle of 22/26°C. RB isolates were sequentially transferred across five host generations using mechanical transmission from a source plant in the previous generation. As a measure of replication, symptoms and

their development rate were recorded every two days post-inoculation for two weeks, and long-term symptom development scored once a week for the following 6 weeks.

Symptoms included: chlorotic or necrotic vein netting, stunting, mottle and discoloration, necrotic lesions, apical tip death, and leaf twisting. Level of recovery was also measured by scoring healthy new leaf growth and uninfected tomato production. Tissue from both symptomatic and asymptomatic plants was serologically tested using DAS-ELISA to confirm infections. An ELISA test was run on the inoculated leaf and new leaf at two weeks post inoculation. Additional ELISAs were run on new leaf tissue at 4 weeks, 6 weeks, and 8 weeks post inoculation to measure symptom progression rate. A final ELISA was run on fruit when they reached maturity around 10 weeks post inoculation.



A.



B.

Figure 3 Symptoms caused by TSWV on tomato (A) necrotic vein netting (B) chlorotic vein netting

Results and Discussion:

This experiment is currently in its final stages. All groups had infections, suggesting that RB isolate transmission is not substantially affected by resistant or susceptible hosts within five generations. However, further analysis of data will determine if there is an impact on pathogen virulence and other aspects of fitness besides transmission rate, including rate of disease spread and proportion of plant tissue infected with the virus. Distinct differences in symptoms were seen between groups (Figure 3). The use of resistant plants in the field may affect the evolution of TSWV, altering virus fitness and virulence. If RB isolates do not suffer a fitness cost, the durability of the *sw-5* gene is not predicted to be high.

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Nourishment and Caste in a Social Wasp, *Polistes metricus*

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The paper wasp *Polistes metricus* (Hymenoptera, Vespidae) is a model organism for studies of the evolution of insect social behavior. Research conducted in the NCSU Phytotron in 2009 continued a multi-faceted research program, previously pursued in part in the Phytotron in 2008, that aims to discover mechanisms of physiology, development, and molecular biology that underlie the differentiation of paper wasps into workers (non-reproductive) and gynes (future reproductives). The main aim of the 2009 research was to generate larvae for microarray analysis of developmental response of wasp larvae to three different feeding treatments. A secondary, but ultimately more productive, objective was to document developmental variables of body size, ovary development, fat content, protein level, juvenile hormone level, and other variables.

Methods

The planned microarray comparison was of larvae reared in captivity on one of three feeding regimens for the foundress: restricted caterpillars (the principal larval food), ad lib caterpillars, and ad lib caterpillars plus daily hand supplementation feeding of fifth-instar larvae. The experimental protocol was to collect pre-foundresses in the early spring when they had just emerged from over-winter quiescence and then to introduce them singly into cages in an environmental chamber in the 'Phytotron' at NCSU. Forty foundresses were set up in this way and divided into the three treatments, with twice the number of foundresses in the restricted food category as in the other two categories. Foundresses that did not initiate nests were replaced with newly-caught pre-foundresses as needed until 40 colonies were under way. As colonies developed, however, it became clear that more than half of the foundresses were not *P. metricus* but were instead a

nearly identical different species. It may be an undescribed species. At that point the 30 or so nest boxes that remained in place from 2007 were checked, and any *P. metricus* foundresses in them were transferred together with their early-stage nests into cages in replacement of the undescribed species. More than half of the colonies were thereby *P. metricus*, although a good number of the undescribed species remained. Daily maintenance became increasingly time consuming as the colonies increased in size, and when the daily hand supplementation began the time requirement became too much for the PI alone. An undergraduate, Talbia Choudhury, was brought into the project at that point. From mid-May until mid-June the daily time requirement for maintenance plus supplementation was typically 8 hours or more.

The protocol was to wait until 3 cocoons were present, then collect a large, near-pupation fifth-instar larva. Many of the restricted nourishment colonies were developing extremely slowly, which led to a slight increase in the feeding rate for them. Due to a combination of the low success of the restricted colonies, in combination with the substantial number of the undescribed species, only four larvae were successfully collected from that treatment. Fortunately, four larvae per treatment is marginally sufficient for an informative microarray experiment. Due to the technical problems with the field-reared specimens in 2007, the 2009 captive-reared specimens have been held in storage at -80C until the technical difficulties have been resolved. Because of the expense and, especially, the enormous time requirement to produce these specimens, they are more-or-less impossible to replace. As stated above, the PI is determined to complete this project successfully.

As the laboratory rearing experiment was being set up in spring 2009, the PI realized that it provided the framework for a study that was envisioned 5 or more years previously. If an experiment was conducted in which food was the only variable, which is impossible in a field experiment, it would be possible to collect data on numerous response variables: time spent in pupation, offspring size, ovarian development in bioassay, protein quantification,

fat quantification, and juvenile hormone quantification. Consequently, in addition to collecting microarray larval specimens from each *P. metricus* colony, all newly-emerged female offspring were collected into one of three analytical categories: two-week bioassay for ovarian development, one-week isolation for full development of JH expression, and immediate collection for protein and fat quantification. A wing measurement will provide size data for all specimens, and pupation time can be determined from daily maps. Data on pupation time, size, and ovary development are in hand. Dr. Timothy Judd of Southeast Missouri State University will be doing the protein and fat assays. Dr. Peter Teal of the USDA in Gainesville, FL, will be doing the juvenile hormone assays.

Presentations of Results

No results of microarray experiments are yet available for presentation.

Preliminary results of the developmental effects of nutrition experiment have been presented in two invited symposium presentations and two invited seminars:

1. J. H. Hunt, 'The mechanisms of (eu)social behavior.' Part of a symposium, 'Understanding insect sociality with Tinbergen's four questions.' Presented on August 24, 2009, at the 31st International Ethology Conference in Rennes, France.
2. J. H. Hunt, 'The origin of castes in social wasps.' Part of a symposium, 'Understanding group living in social invertebrates.' Presented on December 16, 2009, at the National Annual Meeting of the Entomological Society of America in Indianapolis, IN.
3. J. H. Hunt, 'Natural selection and kin selection in social evolution: Paper wasps open the door to understanding.' Presented on November 13, 2009, at the National Evolutionary Synthesis Center in Durham, NC.

4. J. H. Hunt, 'Social evolution: natural selection & kin selection in paper wasps.'
Presented on November 19, 2009, to The Curriculum for the Environment and Ecology at
the University of North Carolina, Chapel Hill, NC.

Three manuscripts are planned for submission for publication from the developmental effects experiment:

1) Developmental variables of individual wasps in response to food levels in a paper wasp, with undergrad Choudhury and collaborators Timothy Judd (Southeast Missouri State Univ.) and Peter Teal (USDA, Gainesville)

2) Colony-level developmental variables of wasps in response to food levels in a paper wasp, with undergrad Choudhury

3) An unusual wasp nest constructed in captivity (a minor note)

Results

The microarray experiment was designed to compare larvae reared in captivity on one of three feeding regimens for the foundress: restricted caterpillars (the principal larval food), ad lib caterpillars, and ad lib caterpillars plus daily hand supplementation feeding of fifth-instar larvae. The experimental protocol was to collect pre-foundresses in the early spring when they had just emerged from over-winter quiescence and then to introduce them singly into cages in an environmental chamber in the 'Phytotron' at NCSU. Forty foundresses were set up in this way and divided into the three treatments, with twice the number of foundresses in the restricted food category as in the other two categories. Foundresses that did not initiate nests were replaced with newly-caught pre-foundresses as needed until 40 colonies were under way. As colonies developed, however, it became clear that more than half of the foundresses were not *P. metricus* but were instead a nearly identical different species. It may be an undescribed species. At that point the 30 or so nest boxes that remained in place from 2007 were checked, and any *P. metricus* foundresses in them were transferred together with their early-stage nests into cages in

replacement of the undescribed species. More than half of the colonies were thereby *P. metricus*, although a good number of the undescribed species remained.

From mid-May until mid-June the daily time requirement for maintenance plus supplementation was typically 8 hours or more. The protocol was the same as for the field-reared colonies in 2007: wait until 3 cocoons were present, then collect a large, near-pupation fifth-instar larva. Many of the restricted nourishment colonies were developing extremely slowly, which led to a slight increase in the feeding rate for them. Due to a combination of the low success of the restricted colonies, in combination with the substantial number of the undescribed species, only four larvae were successfully collected from that treatment. Fortunately, four larvae per treatment is marginally sufficient for an informative microarray experiment.

The Developmental Effects of Differential Nourishment experiment has generated very promising specimens and preliminary data. Food quantity was the only variable. Response variables: time spent in pupation, offspring size, ovarian development in bioassay, protein quantification, fat quantification, and juvenile hormone quantification. Consequently, in addition to collecting microarray larval specimens from each *P. metricus* colony, all newly-emerged female offspring were collected into one of three analytical categories: two-week bioassay for ovarian development, one-week isolation for full development of juvenile hormone expression, and immediate collection for protein and fat quantification. A wing measurement will provide size data for all specimens, and pupation time can be determined from daily maps.

Impact on the Discipline

The PI has long advocated the importance of knowing mechanisms in order to learn the evolution of insect sociality. A *Polistes metricus* EST data set, published in 2007, opened the door to novel studies that delve deeply into the mechanisms that underlie the developmental partitioning of same-generation wasps into workers and gynes. The microarray research proposed and conducted here has taken advantage of this genomic resource to advance knowledge of social insect evolution to new, deeper, and informative

levels that were heretofore inaccessible. A much clearer understanding of the evolutionary process is the result of these researches. The main objective was to rigorously test the hypothesis that the developmental divergence is a consequence of low nourishment leading to worker-biased offspring and high nourishment leading to gynes-biased offspring. A secondary objective was to use the microarray results to identify candidate genes for future research that will be targeted at specific regulatory pathways that could play significant roles in the developmental divergence. A specifically-mentioned possibility for future research of this kind was to identify genes for potential RNAi knockdown studies as a tool for ascertaining the role and importance of those pathways. It is hoped that these objectives will eventually be met and that they will lead to significant advances in understanding and also new avenues for research.

The developmental effects experiment emerged as a bonus from the rearing protocol to generate specimens for the microarray experiment. This experiment is potentially of considerable significance, as it has the potential to tie together heretofore disparate pieces of the mechanisms that underlie the developmental divergence already documented in one publication and in a manuscript now in review. At the same time, the experiment yielded specimens with the potential to add a key missing piece of the puzzle, juvenile hormone. The impact of these summed results is that it is becoming impossible to assert that all *Polistes* offspring have the same development, and in consequence it is becoming increasingly difficult to assert that some offspring altruistically choose to become workers. In other words, a paradigm shift in how we understand insect social evolution is under way.

Training and Development:

Ms. Talbia S. Choudhury participated extensively in the controlled rearing experiment in 2009. In addition to assisting with colony maintenance and hand-supplementation of larvae, she also collected specimens, including hemolymph extractions for protein and juvenile hormone analyses. She is a superb student with a clear focus on advanced training, probably in medical school, although an academic trajectory has become something that she now is considering. She is presently a senior and will be once again

working with the PI in spring semester 2010. This research will be at the bench for DNA extraction and PCR in a study unrelated to the research described in this report. The DNA study is unsupported, and Ms. Choudhury will be working as a volunteer. Ms. Choudhury, a Pakistani-American, shows great promise for a successful and productive career in medicine and/or research.

Influences of Vermicompost on Host Preference and Performance of the Cabbage Aphid Pests *Myzus persicae* and *Brevicoryne brassicae*

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Introduction

Vermicompost has been reported to increase growth and yield for a number of plants, such as, Marigolds, Tomatoes, Peppers, Lettuce, Spinach, Crossandra, and Strawberries (Arancon et al., 2004a, Arancon et al., 2004b, Arancon et al., 2006, Atiyeh et al., 2000, Gajalakshmi and Abbasi, 2002, Gutierrez-Miceli et al., 2007, Peyvast et al., 2007, Zaller, 2007). Interestingly vermicompost has also been shown to increase resistance to pathogen, nematode and arthropod pests (Arancon et al., 2002, Arancon et al., 2005, Arancon et al., 2007, Edwards et al., 2010, Chaoui et al., 2002, Szczech, 1999); however, the mechanism of defense influenced by vermicompost is yet unknown. The objectives of this study were to test the preference and performance of *Myzus persicae* and *Brevicoryne brassicae* on *Brassica oleracea* grown in soilless potting mix amended with various food based vermicompost treatments.

Methods

Plant and insect rearing

Brassica oleracea var. capitata was grown in sun-gro redi earth soilless potting mix amended with 0, 20, 40 and 60% food based vermicompost from Oregon Soil Corporation (OSC). Plants were reared in environmentally controlled growth chambers in the NCSU Phytotron with 14h:10h light: dark at 22° C:20° C respectively. All experiments used plants with 10 true leaves (approximately 1 month after germination). Both aphid species were reared on cabbage plants grown in unamended soilless potting mix; plants were kept in incubators with the same conditions the test plants were reared.

Apterae antixenosis choice assays

One 2.5 cm diameter leaf disc was taken from each treatment and placed in a 9 cm diameter plastic Petri dish on top of agar and filter paper. 10 newly molted apterae adults were then placed in the middle of the dish. The dish was sealed and placed in an incubator and the aphids were allowed to feed for 24 hours; numbers on each disc were counted periodically. This was done for both species separately in this trial as well as the following trials. A total of 24 replicates for each species were recorded.

Alate antixenosis choice assays

One plant from each treatment was placed in a 2ft x 2ft x 2ft mesh and pvc cage located in the NCSU method road greenhouses. Ten newly molted alates were then placed in the center of the cage and allowed to feed for 24 hours. Numbers of aphids on each treatment were recorded at various time intervals as well as total number of nymphs per plant at the end of the experiment. A total of 24 replicates were recorded for *Brevicoryn brassicae*, trials are currently being conducted on *Myzus persicae*.

Antibiosis no choice assays

5 newly molted apterae adults were weighed and then confined to each treatment plant using a sleeve cage made from chiffon mesh; the cage was held off the plant by electrical wire and sealed around the pot with a rubber band. The adults were allowed to feed and reproduce for two weeks; at the end of the two weeks the final mass and total number of adults and nymphs were recorded. A total of 21 replicates for each species were recorded.

Results and Discussion

Performance and preference of both aphid species were effected by vermicompost. For the apterae antixenotic trials there was no antixenotic effect seen for either species; interestingly the specialist *Brevicoryne brassicae* preferred the three vermicompost treatments over the control. There was no difference between the vermicompost treatments however. For both the alate antixenotic trials and the antibiotic trial the 20 % treatments had significantly lower numbers and masses than the control; the 40 and 60% treatments had the same and sometime higher number and masses than the control;

although not significantly higher. For *Myzus persicae* all three vermicompost treatments had significantly lower masses and numbers of adults and nymphs than the control. No results for alata antixenosis for *M. persicae* have been analyzed yet since they are still being conducted. A manuscript of this research is currently being worked on for submission.

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A Phenotype Screen for Potential Gene Candidates to Modify

***Populus* Root Architecture and Function**

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Introduction

With the rise of interest in offsetting carbon emissions with sequestration underground, more knowledge is needed about the pathways of tree root development that facilitate this carbon sequestration. The genus *Populus* has gained much attention recently, because it has been designated as an ideal model species for tree research as a result of its fast growth and small genome size. Recently the genome of one of its member species *Populus trichocarpa* was sequenced (Tuskan et al, 2006), providing the genetic background and tools for a wide range of studies, including determining gene expression that controls tree root development and growth. Extensive research has been carried out on some of the genes involved with root development in *Arabidopsis thaliana*, a small herbaceous plant used as a model organism, but little is known about how similar genes operate in *Populus* roots. To study this question, three genes that play an important role in *Arabidopsis* root meristem development and lateral root initiation, *PLETHORA1* (*PLT1*) (Aida et al, 2004), *SHORT-ROOT* (*SHR*) (Benfey et al, 1993), and *NAC1* (Xie et al, 2000), were identified as putative homologous genes in *Populus*. The gene expression of each gene of interest was modified either through inserting an inverted repeat to cause RNA interference (RNAi) (Fire et al, 1991) to create an under-expression construct, or by inserting an extra copy of the native gene to create an over-expression construct. Three RNAi constructs were made, which we call *PLTi*, *SHRi*, and *NACi*, and a single over-expression transgenic construct was made, which we call *SHR+*. The purpose of this study was to screen for altered shoot and root phenotypes in each of the genetic constructs in comparison to the wild type, and to see if water or nutrient stress would bring out altered phenotypes that were not observed in tissue culture.

Methods

Plant Material

The genome of *P. trichocarpa* was used to identify the gene sequences, but the test species used was *P. tremula* x *P. alba* hybrid clone INRA 717-1B4, which is a hybrid of European Aspen and White Cottonwood, and is more easily maintained as a clone in tissue culture. A preliminary phenotype screen was carried out in tissue culture, and from this data a selection of independently transformed lines from each genetic construct was chosen for further observation in the growth chamber. Twenty-three genetic lines (4 *PLTi*, 5 *SHRi*, 8 *SHR+*, and 6 *NACi*) each having 16 individual ex-plants as well as wild type trees (WT 717) were transferred from tissue culture into Ray Leach Supercells (Stuewe & Sons Inc., Tangent, OR) filled with river bottom sand.

Environmental Conditions and Plant Acclimation

A walk-in controlled environment growth chamber in the NCSU phytotron was used with a long day (16 hour) photoperiod and a constant temperature of 20° C. A high humidity environment was created to acclimate the plants by using a misting system enclosed in plastic sheeting. Over the four-week acclimation period the humidity level was gradually decreased from 5 seconds of water every 5 minutes to 5 seconds every 15 minutes, and the light level was gradually increased from ~150 mmol/m²/s⁻¹ to ~600 mmol/m²/s⁻¹ by removing layers of shade cloth.

After the acclimation period, and once the roots began to reach the bottom of the super cells, the trees were transferred to 4" x 4" x 14" tall-one tree pots (Stuewe & Sons Inc., Tangent, OR) also filled with river bottom sand and grown under the same photoperiod and with day/night temperatures of 22°/ 20° C. The trees were given de-ionized water twice a day until flow-through and nutrients once a day on Mon/Wed/Fri for two weeks.

Experimental Design

Once the trees were established in the larger pots, they were set up in a split-plot experimental design with four blocks. Each of the blocks had a complete 2x2 factorial design with treatments for high and low water and high and low nutrients. One individual of each of the 23 genetics lines plus 5 individuals of WT were randomized within each treatment plot. The high and low water treatments were maintained

throughout the experiment so the high treatment was twice the amount of the low treatment, but the actual amount of each increased throughout the experiment as the trees required more water. The low nutrient treatment was a 1/3 dilution of the regular Phytotron nutrient solution applied three days per week in the morning, and the high nutrient treatment was the regular strength Phytotron nutrient solution applied 6 days per week in the morning.

Physiological Measurements

Measurements of the height, diameter at half of the height, and the number of leaf nodes were taken at the start of the treatment application and at weeks 1, 3, and 5. After 8 weeks, each block was harvested in succession one week apart. During the harvest, the final height, base diameter, diameter at half of the height, number of leaf nodes, leaf count, and total leaf area were recorded. The stem and leaves were then separated and the roots washed of sand and rocks. The leaves, stem, and roots were dried to a constant weight and total biomass for each section was recorded.



Figure 1. Different stages in the growth process of hybrid poplar in our experiment

Results

This study showed there was a large difference in shoot and root growth between trees in the low vs. high nutrient treatments, but there was not much difference between trees in the low vs. high water treatments (Figure 2, panels a & b). Instead of adapting their growth rate, trees in the low water, high nutrient treatment plots would wilt under water stress and either recovered quickly after being watered or in a few cases they would drop their leaves and then re-sprout new branches. The other growth characteristics measured such as height, base diameter, and leaf area (data not shown) also showed similar separation among treatments as the shoot and root biomass.

The over-expression genetic construct *SHR+* was the only construct that showed an altered shoot and root phenotype. *SHR+* had an average of about half as much shoot and root biomass compared to wild type regardless of the treatment (Figure 2, panel c). *SHR+* lines also displayed varying degrees of curled or deformed petioles and leaves (Figure 3). We did not detect any different phenotypes emerging in the water or nutrient stressed trees.

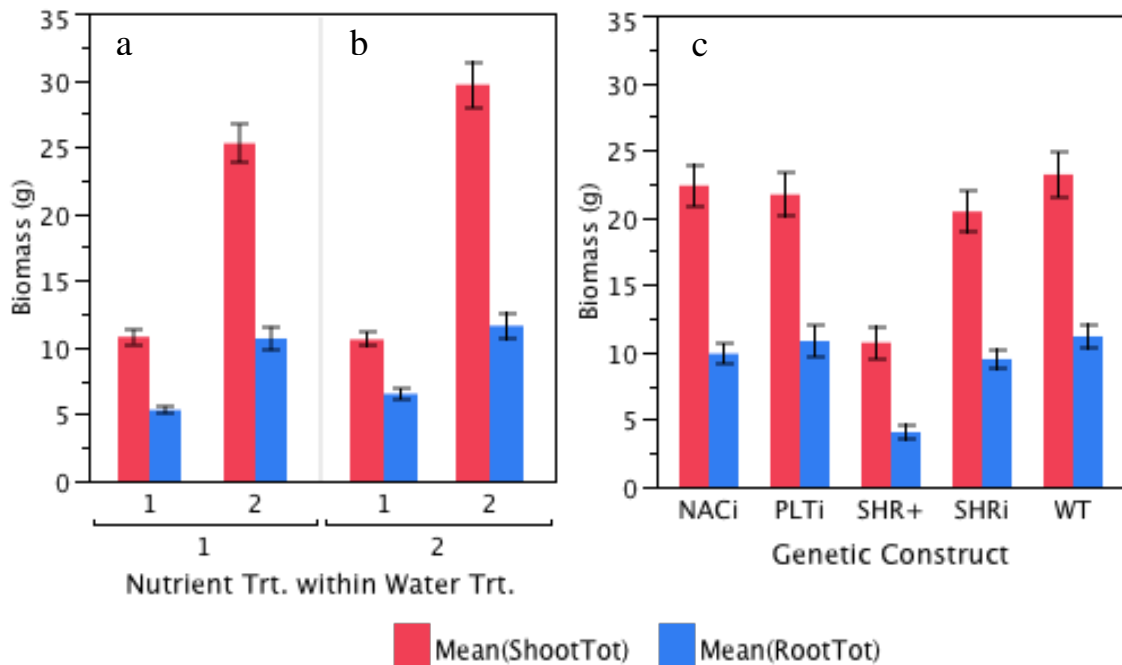


Figure 2. The mean \pm std. error of shoot and root total biomass for a.) low water plots with low(1) and high(2) nutrients, b.) high water plots with low(1) and high(2) nutrients, and c.) each genetic construct averaged across treatment plots.



Figure 3. A normal wild type leaf (a) compared to the deformed leaves (b), and curled leaves (c) of two different *SHR*⁺ lines.

Conclusions

The lack of visible phenotypes in the three RNAi constructs (*NACi*, *PLTi*, and *SHRi*) suggests that either these knocked-down genes do not have the same function as observed in *A. thaliana*, or there are other genes with similar function that are complementing the function of the one we knocked-out. The next step will be to characterize the gene expression levels of each gene construct and try to detect genes in the same family that could have similar functions. The stunted growth and curled leaves of the *SHR*⁺ over-expression construct show that adding copies of the *SHR* gene has an effect on the growth and development of the plant, but in some ways that we did not expect. Since the strong CaMV 35S promoter was used the *SHR* gene could be ectopically expressed in tissues where it would not normally be. *SHR* is a transcription factor so it could have a large effect on other genes downstream. Further research is required to determine which other genes are being affected.

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Development of an *A. thaliana*-Based Screening Assay to Identify

Pathogenicity Genes in *Aspergillus flavus*

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Introduction

Aspergillus flavus infects developing seeds of corn, peanuts, cotton and tree nuts and contaminates them with the carcinogen aflatoxin. Its ability to attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground, show that this fungus has evolved an armory of weapons that allows it to breach the biochemical barriers responsible for non-host resistance. Few plant pathogenic fungi have such a broad host range and the ability to render a crop unmarketable.

Unfortunately, little is known about pathogenicity factors in *A. flavus*. Only one gene has been shown to be conclusively associated with virulence in *A. flavus*. To gain a better understanding of pathogenesis in the *A. flavus*/maize interaction, gene expression during the infection of developing maize seeds was monitored in the field over the last two growing seasons in North Carolina using a custom Affymetrix GeneChip DNA microarray. This array contains elements representing approximately 13,000 *A. flavus* genes and 8,000 maize genes previously shown to be expressed in seeds.

Transcriptional analysis identified 481 genes to be significantly more highly expressed in the fungus when grown on living maize kernels rather than on autoclaved kernels at the same developmental stage. Of these 481 genes, 166 were expressed at least 2 fold higher on living kernels. We hypothesize that within this set of genes are genes necessary for pathogenicity of *A. flavus*.

The overall research goal is to identify genes in *A. flavus* necessary for pathogenicity. The specific goal of this project is to develop a disease-screening assay in *Arabidopsis thaliana* that will allow the identification of candidate pathogenicity genes in *A. flavus*. *Arabidopsis thaliana* is not a known host for *A. flavus*, but Hammond et al. (2007) showed that *A. flavus* can colonize *A. thaliana* seeds. We propose to test seeds as well as leaves in assays to evaluate the pathogenicity of *A. flavus* mutants with deletions

for candidate pathogenicity genes. The reliability of the assay will be evaluated based on its ability to discriminate between a wild type strain and a deletion mutant lacking a gene for Cu/Zn superoxide dismutase. We have determined that this gene is required for full pathogenicity in *A. flavus*. The specific aims of this proposal are: **1. Develop an *A. thaliana*-based screening assay identify pathogenicity genes in *A. flavus*; 2. Validate the screening assay using a known pathogenicity mutant of *A. flavus*.** *Arabidopsis thaliana* is well suited for this assay because it is easy to grow and adaptable to large-scale screening assays. Furthermore, it is a model research plant with well-characterized mutants in pathogen defense pathways. Thus, the development of an *A. flavus*/*A. thaliana* pathosystem will enhance future research on host resistance to *A. flavus*.

Materials and Methods

Arabidopsis thaliana- Plants were grown from seed at the NCSU Phytotron in a controlled growth chamber at a constant temperature of 20° C while receiving eight hours of incandescent plus fluorescent light per day. Plants were watered every other day with deionized and once a week with nutrient rich water.

Plant Lines

2-8 - Transgenic line expressing the mammalian type 1 inositol polyphosphate 5 phosphatase (InsP 5-ptase). (Perera et al., 2006 and 2008)

SOR 3 and 9 - Transgenic line expressing the superoxide reductase (SOR) gene from the archaeal hyperthermophile, *Pyrococcus furiosus*. Im et al., 2005)

Columbia – wild type

GFP - wild type expressing a green fluorescent protein.

Aspergillus flavus- Fungal strains from stocks were streaked on plates of Potato Dextrose Agar (PDA) and incubated for seven days. To prepare agar plugs for inoculation, spores of the fungus were removed from the cultures and added to molten agar to a final concentration of $1e^6$ conidia/ml.

Fungal Strains

SOD - Δ sod mutant with reduced SOD activity

nepA - Δ nepA mutant lacking the ability to produce a necrosis inducing peptide

3357 - wild type

3357-5(+)pyr - wild type that has undergone a control transformation

AFC-1++ - wild type

***A. thaliana* Inoculations**- Agar plugs (0.8% agar and 5 in. diameter) embedded with *A. flavus* were placed on top of *A. thaliana* leaves within a large petri dish containing Murashige and Skoog (MSR) media. The dishes were taped shut and left for five days.

Results

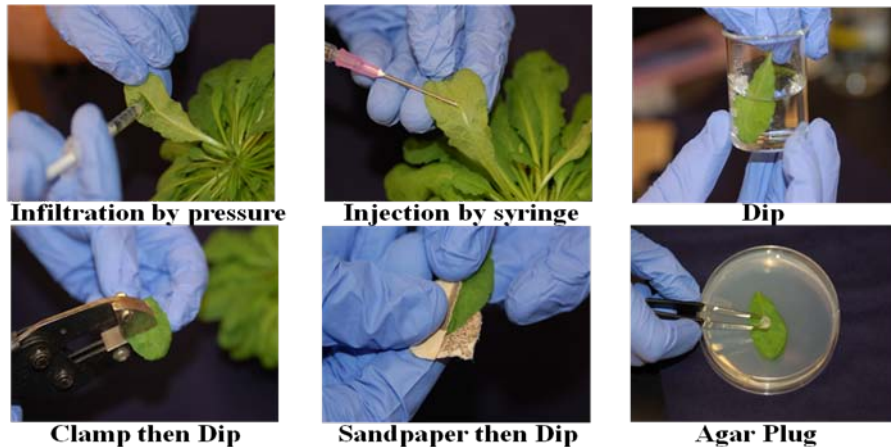


Figure 1. Test of different inoculation methods- Several techniques were used to inoculate *A. thaliana* with *A. flavus*. Based on consistency, ease of manipulation, and reproducibility, we judged agar plugs to be the optimal method of inoculation.



Figure 2. Analysis of Lesion Size- Two methods were used to measure lesion size on *A. thaliana* leaves. A manual method using a ruler (left) was used to measure length and width of a lesion in millimeters. The second method (right) involved APS ASSESS imaging software to calculate the lesion as a percent of the overall area of a leaf. White lines denote boundaries of lesion and uninfected areas.

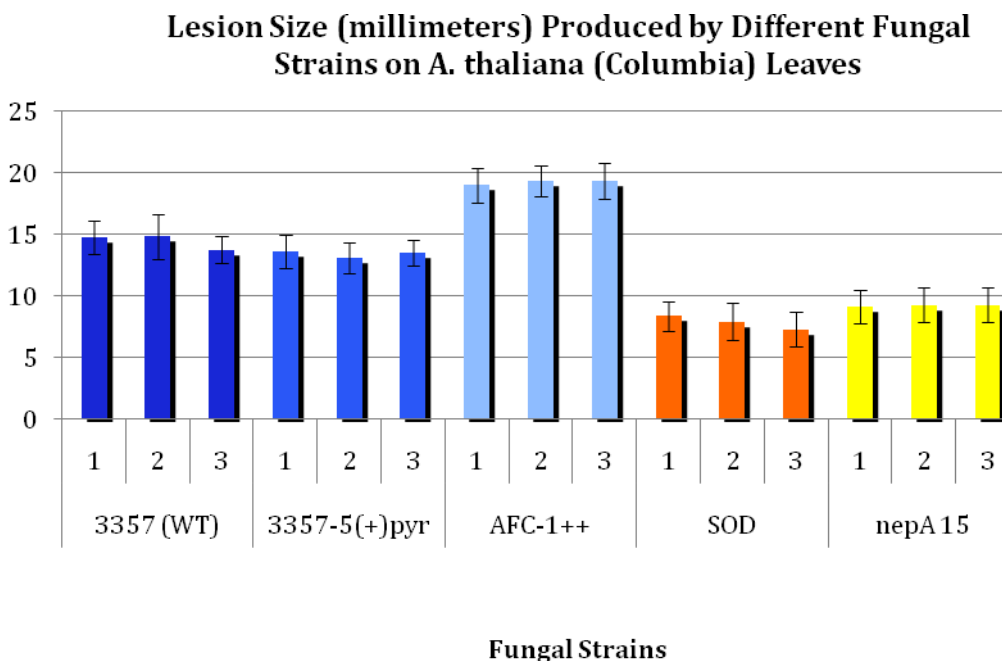
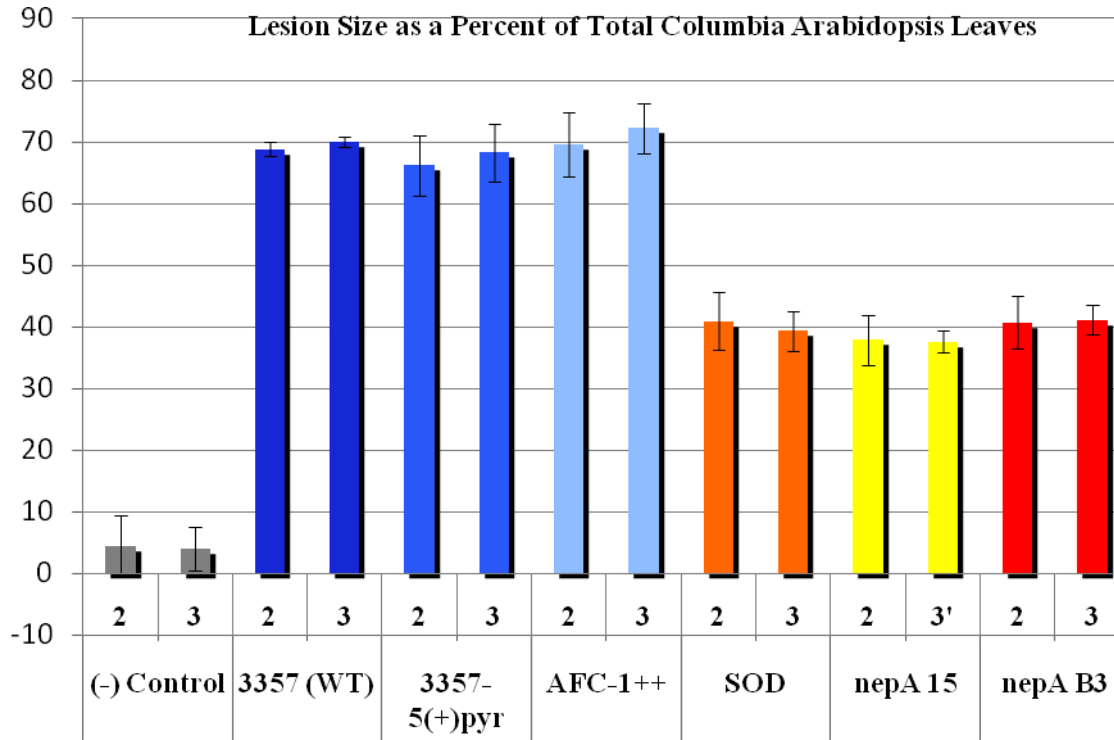


Figure 3. Significant lesion size variation between fungal controls and mutants on *A. thaliana* (Columbia). 480 *A. thaliana* (Columbia) leaves, collected from 48 *A. thaliana* (Columbia) plants, were used in three biological replications. These replications revealed significant lesion size differences between *A. flavus* controls and mutants. *A. flavus* control strains produced larger lesions than either of the *A. flavus* mutant lines. A p-value less than .0001 revealed that *A. flavus* control and mutant effects are significantly different on *A. thaliana* (Columbia).



Fungal Strains

Figure 4. Lesion size was significantly smaller with *A. flavus* mutant strains. Three biological replications were carried out involving 320 *A. thaliana* (Columbia) leaves from 640 *A. thaliana* (Columbia) plants. Replication 1 was not included due to large scale contamination that significantly skewed observations and results. On replications 2 and 3 *A. flavus* controls represented the largest lesions while *A. flavus* mutants had significantly smaller lesions. The *A. flavus* controls produced a 28% larger lesion than the *A. flavus* mutant strains. A p-value less than .0001 showed that *A. flavus* control and mutant effects are significant within the three replications.



Figure 5. Photograph showing representative leaves five days after inoculation with fungal strains used in Fig. 4.

Fungal Lesions on Different Arabidopsis Lines

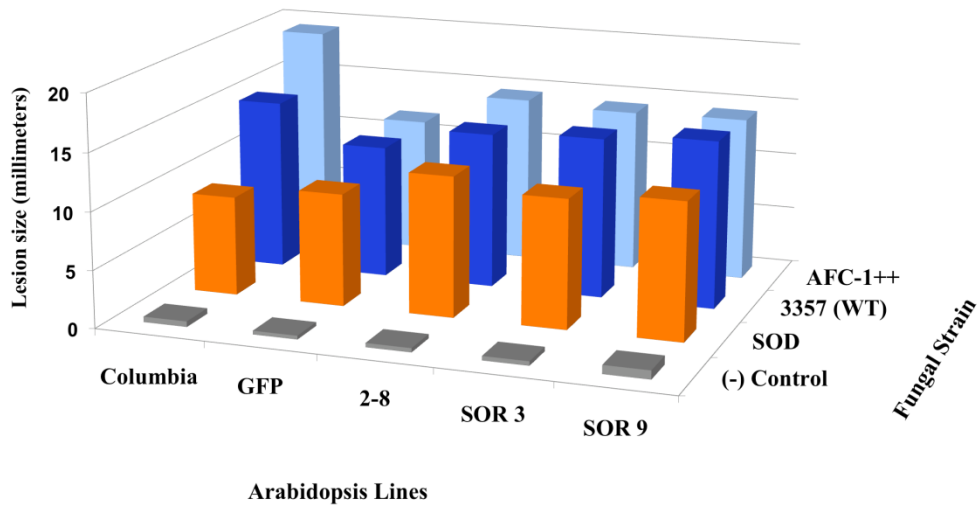


Figure 5. The capacity to produce or resist reactive oxygen species affects pathogenesis of *A. flavus*. Six biological replications were conducted on Columbia, GFP, and 2-8 lines of *A. thaliana* and three biological replication were performed with both SOR3 and SOR 9. Transgenic line 2-8 has impaired lipid signaling and altered responses to different stresses. Lines SOR3 and 9 are 2 independent transgenic lines expressing the *P. furiosus* SOR gene and have a higher capacity to deal with ROS. Each replication had a total of 32 different *A. thaliana* plants for a total of 192 *A. thaliana* plants for the experiment. *A. flavus* controls produced a larger lesion than *A. flavus* mutants on all lines of *A. thaliana*. A two-way ANOVA test showed that the interaction effect between plant and fungus is significant.

Conclusions

SOD, nepA 15. and nepA B3 represent *A. flavus* mutants that have deletions for putative pathogenicity genes in the fungus. All of these fungal strains produced significantly smaller lesions on *A. thaliana* than the controls. Thus, this assay reliably identified strains of the fungus impaired in pathogenicity. The studies comparing the susceptibility of different lines of *A. thaliana* showed Columbia to be the most susceptible to the *A. flavus* pathogenic strains AFC-I ++, 3357, and 3357-5(+) pyr). Lines 2-8, SOR 3 and SOR 9, however, were more susceptible to fungal strain SOD than was Columbia. The SOD mutant was created by deleting a gene coding for a copper/zinc super oxide dismutase. SOD mutants are impaired in their ability to tolerate reactive oxygen species (ROS), and less able to defend themselves from ROS produced during plant defense. The

ability of the SOD mutant to grow better on SOR 3 and SOR 9 is likely a function of the altered status of ROS in these plants. SOD mutants can grow somewhat better on these plants because they accumulate less ROS. It is unclear why line 2-8 is more susceptible to the SOD mutant, but this line is also compromised in the host defense response. Both the 2-8 mutation and the SOR mutation may be very helpful in understanding host resistance to *A. flavus* as they appear able to detect subtle differences in the pathogenicity of *A. flavus*. Results from these studies show the potential use of an *A. thaliana* screening assay to identify mutants of *A. flavus* impaired in pathogenicity. Such an assay will greatly facilitate functional genomic analysis in *A. flavus*. Current screening assays require immature maize kernels, which are not available in quantity except during the growing season. Further, the ability to study disease on the genetic model *A. thaliana* with well-characterized mutants in host defense will help understand diseases caused by this opportunistic pathogen.

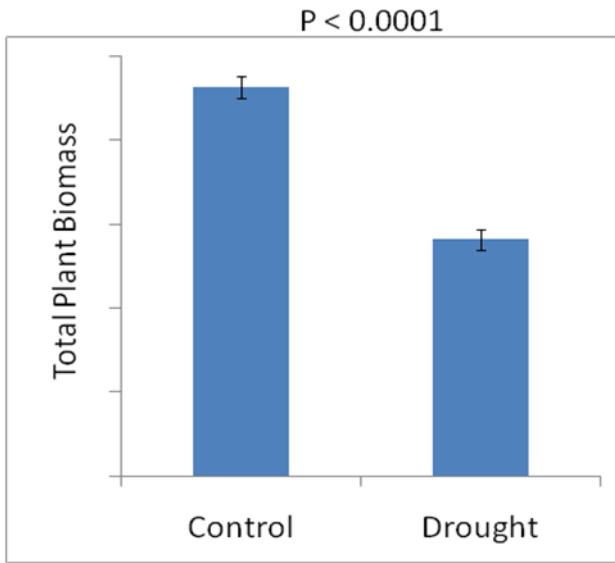
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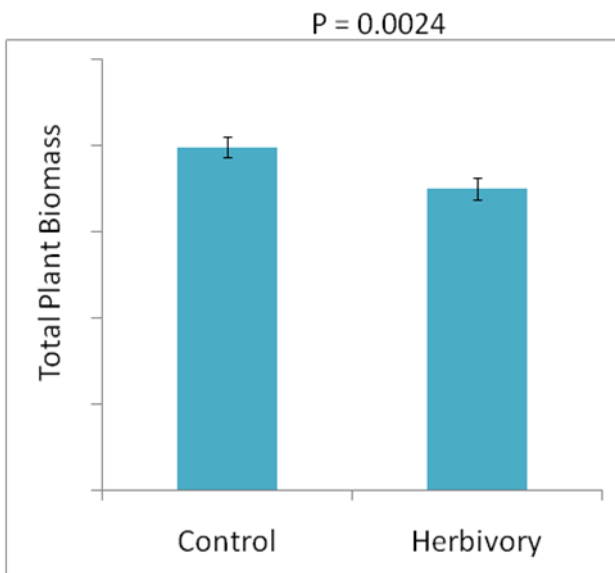
The Effects of Climate Change on Plant – Herbivore Interactions in Soybeans

Rose Grinnan and Marc Johnson

Global climate change is predicted to cause increased temperature and drought, as well as an increase in herbivorous insect populations. Currently, we have little understanding of how plants, especially our agricultural crops, will respond to such changes. We pose the following questions: 1) Will increases in temperature and drought affect plant resistance to herbivory and 2) How do the combined effects of temperature, drought and herbivory affect plant performance? To address these questions we used soybean (*Glycine max*, cultivar *NC-Roy*), an important agricultural crop. In growth chambers we experimentally manipulated temperature, water and herbivory in a fully factorial design to understand how these factors influence plant performance. We found that drought had significant negative effects on above- and below-ground plant biomass, and the interaction of drought and herbivory had significant effects on root: shoot ratios. An increase in temperature increased the rates of germination, growth, and the negative effects of drought and herbivore damage. We plan to use these results to design a field experiment that will clarify the importance of these stress factors in real environmental scenarios. However, from this research, we do conclude that the predicted climate change will increase the potential for stress related damage to soybeans and that it would be beneficial to explore new varieties of soybeans that can withstand increased levels of drought and herbivory in light of global temperature change.



39% decrease



12% decrease

Functional Characterization of a Gravity-Regulated Sterol-Binding Protein *Rosy1* in *Arabidopsis thaliana* Roots

Dr. Heike Winter-Sederoff and Jyoti Kajla

Introduction

Plants use light and gravity to orient their direction of growth. Roots grow towards the vector of gravity to anchor the plant in the soil and find water and nutrients. Changes in environmental conditions cause changes in gene expression that affect the plants' response. We identified gravity-induced fast and transiently up-regulated transcripts in *Arabidopsis* root apices (Kimbrough et al. 2004). One of these transcripts, for gene *rosy1*, is a root specific MD2 lipid binding domain containing protein. Further characterization of *rosy1* showed that it is also regulated by in response to directional light with the same kinetics as its response to gravity (Salinas-Mondragon et al. 2005). Computational analysis showed the protein ROSY1, has a high structural similarity to protein NPC2, malfunctioning of which causes the lethal Niemann Pick disease in human infants. The recombinant protein ROSY1 binds with to the plant sterols stigmasterol and sitosterol, and with a lower affinity to cholesterol. Immunolocalization shows that ROSY1 is localized in vesicular membranes at the root tip. Enhancer trap mutants for the gene show a severe growth phenotype. Preliminary data show that while the mutant roots bend faster in response to gravity stimulation, the shoots show a delayed bending response. Further experiments to understand the role of this protein in plants, and its associations with other plant proteins are underway. (This work is supported by NASA).

Materials and Methods

- 1.) Phytotron space was used to grow knock-out mutant *rosy1-1* and study its growth phenotype.

Soil Preparation

Soil bags were opened in the phytotron and insecticide Marathon was added to the soil (1.82g/L). Pots that were pre-washed with 10% Chlorox solution followed by double distilled water, were taken to the phytotron and filled with soil.

Plant Preparation

Wild type *Arabidopsis* seed as well as *rosy1-1* knock-out seed were sterilized and grown on 1X MS medium for 10 days, and then taken to phytotron and planted in pots. Growth was observed.

Results:

The knock-out mutants showed considerable, but not consistent growth variation as compared to the wild type plants.



Conclusion

To study the phenotype of the knock-out mutants, a continuous batch of seed will be grown in the phytotron and growth will be monitored at regular time intervals.



P.S. KO At2g16005 is the *rosy1-1* knock-out mutant in these pictures.

2.) Phytotron space was used to grow wild type and *rosy1-1* plants for transformation

Expression Analysis

To study in which tissues the promoter was turned on in the plants, wild type plants were used for transformation with promoter:GFP construct. The plants were grown in the phytotron up till they were 21 days old and showed the first inflorescence. After that the plants were moved out of phytotron for transformation and seed set.

Protein Analysis

To study the intracellular localization in steady state and gravity stimulated plants, and to rescue the growth phenotype in *rosy1-1* plants, the plants were transformed with ROSY1:GFP constructs. The plants were grown in the phytotron up till they were 21 days old and showed the first inflorescence. Then the plants were moved out of phytotron for transformation and seed set.

Conclusion

Until transformation experiments work, a continuous batch of wild type and *rosy1-1* plants will be grown in phytotron up till they are 21 days old.

Carpogenic Germination of *Sclerotinia minor*

J.E. Hollowell and B.B. Shew

INTRODUCTION

Sclerotinia blight caused by *Sclerotinia minor* Jagger is a serious disease of peanut (*Arachis hypogaea* L.) in North Carolina, Virginia, Texas, and Oklahoma, resulting in large yield and economic losses. *Sclerotinia* is a soilborne fungus characterized by white fluffy mycelium and small (0.5-2.0 mm) irregular sclerotia found in soil and on organic plant debris. Sclerotia most commonly germinate myceliogenically and infect available susceptible peanut plant tissue near the soil surface.

Sclerotia can also germinate carpogenically, forming apothecia and releasing ascospores for infection. However, carpogenic germination was thought to be rare and not important on peanut because it occurs in late winter or early spring before peanuts are planted.

In recent years *Sclerotinia minor* has been found infecting weed hosts and producing apothecia in fallow peanut fields in late autumn after the peanut crop has been harvested. This suggests that weed infection by ascospores may support populations of *S. minor* between peanut crops. Previous reports indicate a conditioning process is necessary for differentiation of the apothecia.

Experiments in the phytotron were conducted to identify differences in apothecial formation by isolates of *S. minor* found infecting weed hosts from fallow peanut fields.

MATERIALS AND METHODS

Sclerotial production. Isolates of *S. minor* originally collected from twenty different weed hosts and one from peanut were observed for apothecial production (Table 1). The isolates were cultured on sterile oat grains, dried, and stored in Nalgene bottles in the refrigerator.

An oat grain of each isolate was placed on potato dextrose agar (Pda) until mycelium emerged (2-3 days). Each isolate was then transferred to fresh Pda and allowed to grow for 2 days.

Isolates were grown on carrot discs to produce sclerotia >2 mm diameter. A volume of 150 cm³ of unpeeled carrot discs sliced 6 mm thick were placed in a 250 ml flask with 5 ml of deionized water and autoclaved. Five mycelial plugs (each 4 mm) were added to the carrot discs and incubated in the dark at room temperature for 10 days without shaking.

Once black sclerotia had formed and white initials were no longer present, water was added to a 250 ml volume and flasks were shaken to dislodge the sclerotia. The suspension was poured over a 850 µm sieve nested on a 600 µm sieve to separate the sclerotia. Hyphal tissue was removed with the assistance of hand tweezers. Sclerotia were air-dried on paper towels for 48 h and separated by size on >2 mm and >1 mm sieves. Sclerotia >2 mm were used for apothecia production. Weights of the sclerotia from each isolate were recorded by size separation and in groups of 10 for those used in the jars.

Stipe and apothecial production. Coarse sand and deionized water (4:1 v/v), were prepared in 120 ml glass jars and autoclaved for 45 min. Ten sclerotia from each fungal isolate were placed on the sand surface in the jars. The jars were covered with sterile small plastic petri dish lids (60 x 15 mm) and sealed with Parafilm. The sclerotia were placed in a consistent pattern so they could be repeatedly evaluated over time. Jars were arranged in a randomized complete block with 3 reps per isolate.

A three-stage conditioning process was used to induce production of apothecia. The sclerotia were incubated at 4°C in the dark for 30 days, checked for mycelial germination, and then moved to 12°C. After 14 days, sclerotia were checked for stipe formation. Jars remained at 12°C until 50% of sclerotia in a jar had stipes 5 mm long. After 62 days all jars were moved to a growth chamber at 17 ± 2°C with 12 h of diurnal light cycle under low intensity lights until apothecia were fully developed. Formation of stipes and differentiation of apothecia were observed under the dissecting scope weekly for 12 weeks. The study was repeated in different chamber locations.

Ascospore evaluation. After 30 days of light treatment, a subset of apothecia bearing mature asci was removed from the jars. Apothecia were placed on their sides on blue medium to test ascospore release. The blue medium consisted of 30 g Pda, 20 mg PCNB (75% WP), and 50 mg pH indicator Bromophenol blue per liter deionized water. A yellow halo indicating the presence of ascospores aided in the microscopic observation. Single ascospores were transferred to Pda to confirm culture growth.

Seven jars with apothecia were placed near six-week-old peanut plants in the greenhouse and apothecia allowed to release ascospores. Peanut plants growing in 15 cm pots were bagged individually with a jar and a plate of blue medium. The blue medium was used to collect ascospores, indicating release from the apothecia. *Sclerotinia minor* isolations were made from separate plant locations exhibiting symptoms of infection.

RESULTS AND DISCUSSION

Stipes were produced from 16 of 21 isolates and 14 isolates produced asci although stipes of one isolate died (Table 1). Not all of the isolates produced stipes and asci in this experiment. However, some of isolates not producing stipes and asci in this study have produced them in previous experiments. Fifty-five of 60 sclerotia from the cudweed isolate produced at least one stipe. For the isolates that produced stipes, the overall average time to first stipe appearance ranged from 64 to 113 days. Isolates from cudweed and Pennsylvania everlasting produced the most stipes. For the isolates that produced asci and ascospores, the average time to the first ascus formation ranged from 87 to 106 days. Sclerotia of the cudweed isolate produced the highest proportion of fertile apothecia. Thirteen of the isolates had 50% or more of the stipes producing asci and ascospores. Among isolates that produced numerous stipes, the spiny sowthistle isolate had the greatest percentage of stipes producing asci and ascospores with 98%. Sclerotia of several isolates germinated myceliogenically in addition to producing stipes and asci.

Correlation of sclerotial weight with proportion of sclerotia producing at least one stipe with number of stipes per sclerotium and with number of stipes with asci per sclerotium was not significant $r = -0.09$ to $r = -0.05$ (NS).

Jars containing apothecia from the wild mustard and the small flowered bittercress isolates released ascospores when bagged with peanut plants, as evidenced by spores growing on the blue medium. Separate infection sites were observed on these peanut plants, indicating that independent ascospores infected the plants. It is possible the other jars of apothecia unsuccessfully infected plants because there was no ascospore release observed, they were past maturity at the time of release, or those plants were resistant to infection.

The majority of the isolates of *Sclerotinia minor* observed were capable of producing stipes and apothecia even though there were differences in the numbers produced. It is possible that infection of weed species and apothecial production may support populations of *S. minor* between peanut crops.

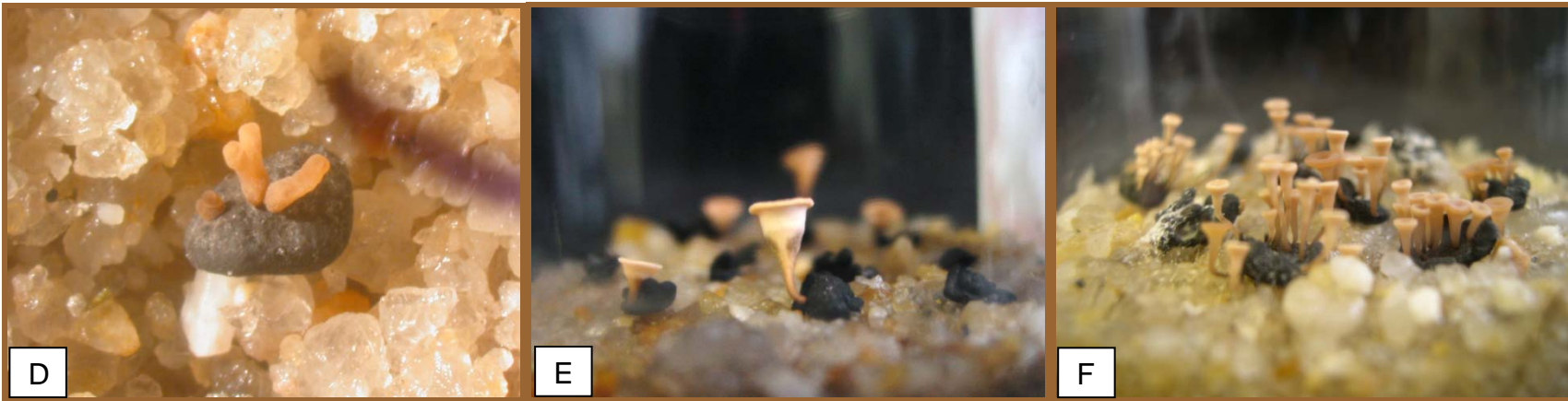
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Cubeta, M. A., Sermons, D. N., Cody, B. R. 2001. Mycelial interactions of *Sclerotinia minor*. Phytopathology 91: S19. Publication no. P-2001-0138-AMA.

Isolate #	Host Name	Scientific Name	Average time to first stipe	Average time to first ascus	Percentage of stipes producing asci	Number of stipes produced per 60 sclerotia
P-13	Peanut	<i>Arachis hypogaea</i>	79	92	60	7
U-1	Annual bluegrass	<i>Poa annua</i>	73	87	89	43
W-1	Yellow nutsedge	<i>Cyperus esculentus</i>	--	--	--	0
W-8	Mouseear chickweed	<i>Cerastium vulgatum</i>	113	--	0	1
W-10	Cutleaf eveningprimrose	<i>Oenothera laciniata</i>	68	95	77	28
W-11	Horseweed	<i>Conyza canadensis</i>	82	96	77	24
W-12	Wild mustard	<i>Brassica kaber</i>	83	99	93	39
W-17	Swinecress	<i>Coronopus didymus</i>	--	--	--	0
W-26	Henbit	<i>Lamium aplexicaule</i>	106	106	100	1
W-31	Small flowered bittercress	<i>Cardamine parviflora</i>	90	95	73	18
W-34	Mouseear cress	<i>Arabidopsis thaliana</i>	84	92	69	39
W-37	Common chickweed	<i>Stellaria media</i>	--	--	--	0
W-38	Carolina geranium	<i>Geranium carolinianum</i>	--	--	--	0
W-41	Eclipta	<i>Eclipta prostrata</i>	--	--	--	0
W-42	Field garlic	<i>Allium vineale</i>	64	--	--	0
W-45	Prickly sida	<i>Sida spinosa</i>	87	92	100	3
W-47	Ivy-leaf morning glory	<i>Ipomoea hederacea</i>	76	88	79	16
W-48	Hairy bittercress	<i>Cardamine hirsuta</i>	84	99	43	7
W-50	Cudweed	<i>Gnaphalium uliginosum</i>	69	89	77	55
W-51	Pennsylvania everlasting	<i>Gamochaeta pennsylvanica</i>	75	92	61	46
W-53	Spiny sowthistle	<i>Sonchus asper</i>	80	97	98	33



- A. Isolate grown on carrot discs to produce sclerotia
- B. Sclerotia separated by size > 2 mm and > 1 mm
- C. Apothecium placed on side on blue medium to test ascospore release



- D. Stipe initiation from sclerotia conditioned at 4°C and then 12°C
- E. Ascus formation on end of stipe
- F. Multiple asci formation of sclerotia

Effect of Temperature on Expression of Soybean Chloroplast Fatty Acid Desaturases GmFAD7 and GmFAD8

Robert G. Upchurch and Martha E. Ramirez

Our laboratory focuses on the effects of abiotic and biotic (pathogen) stresses on growth, development, fatty acid metabolism, and defense responses in *Glycine max*. Temperature stress, especially cold stress is known to affect chloroplast membrane structure and lipid composition in *Arabidopsis thaliana*. Previously, we cloned and sequenced two nuclear encoded, chloroplast localized omega-3 fatty acid desaturase genes, *GmFAD7* and *GmFAD8* from cultivar Dare. We suspected that *GmFAD8* expression (transcript accumulation) would be up-regulated and *GmFAD7* would not be regulated by cold temperature as has been demonstrated for the *Arabidopsis AtFAD8* and *AtFAD7*. We were granted the use of three Phytotron HID C chambers (June 01-July 30, 2009) for an experiment to determine the effects of temperature on expression of *GmFAD8* and *GmFAD7*.

Glycine max maturity group V cultivar Dare seeds were pregerminated and planted to a density of one plant per 15 cm diameter pot containing standard growth medium. A chamber irradiance level of approximately $650\mu\text{E}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ was maintained throughout plant growth. From day 1-28, all 24 plants were grown in a chamber with a D/N = 26/22°C and D/N = 12/12 h. On day 29, eight plants were transferred to and grown (through day 36) in a chamber with settings of D/N = 32/28°C, 12/12 h; and eight plants were transferred to and grown in a chamber with settings of D/N = 20/16°C, 12/12 h. Eight plants remained in the first chamber at D/N = 26/22°C and D/N = 12/12 h for the fifth and final week of the experiment. A daily watering regime of 1x nutrient and 1x deionized water was maintained. During week five, leaves were harvested at 0, 6, 12, 24, 48, 72, and 96 hours after the redistribution of the plants to the chambers. Detached leaves at these sampling times were placed in screw-top polypropylene tubes and immediately frozen in liquid nitrogen. Leaf samples were placed in the -80°C ultralow freezer for storage until processing for RNA extraction and fatty acid analysis.

A trial analysis of the effect of temperature on *GmFAD8* expression was performed on leaf samples collected at 72 h into the growth temperature manipulation. Briefly, frozen leaves were

ground to a powder in liquid nitrogen with mortar and pestle and extracted for total RNA using the RNeasy mini plant RNA extraction supplied by Qiagen (Valencia, CA). Traces of genomic DNA were eliminated by treatment of the RNA with the on-column DNase digestion system from Qiagen. RNA yield and quality were estimated by measuring absorbance at 260 and 280 nm with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). First strand cDNA synthesis was carried out using the High Capacity cDNA Reverse Transcription kit with MultiScribe reverse transcriptase (RT) and random primers (Applied Biosystems, Foster City, CA). qRT-PCR assays with SYBR green were performed in triplicate with an i-Cycler thermocycler (BioRad, Hercules, CA) using *GmFAD8*- and *Soy57* actin-specific primers. Absolute quantification of transcript abundance was calculated using standard curves obtained for both genes. C_T values and i-Cycler software were used to plot a standard curve that allowed quantification of the target gene in each sample. Data from *Soy 57 actin* values were used to normalize *GmFAD8* expression.

Values of normalized *GmFAD8* transcript accumulation were: for the cold temperature (20/16°C), 1875; the normal temperature (26/22°C), 557; and for the warm temperature (32/28°C), 400. These measurements suggest that a relatively cold temperature up-regulates soybean *GmFAD8* expression as was previously documented for this chloroplast localized gene in *Arabidopsis*. Processing of all leaf samples is now underway and all leaf RNAs will be analyzed for *GmFAD8* and *GmFAD7* transcript abundance using qRT-PCR. In addition, the fatty acid composition of the chloroplast membrane lipids will be analyzed from the leaf samples.

Effect of Temperature on Latent Period of *Stagonospora nodorum* Blotch in Winter Wheat under Field Conditions

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Abstract

Stagonospora nodorum blotch (SNB) is caused by *Stagonospora nodorum* (teleomorph = *Phaeosphaeria nodorum*) and yield losses from severe disease epidemics can be as high as 50%. To establish a model for SNB development based on the effects of temperature on the latent period relative to the host, batches of two winter wheat cultivars (AGS 2000 and USG 3209) were inoculated with pycnidiospores of *S. nodorum* at weekly intervals from February 2009 to June 2009. After 72 h of incubation, plants were exposed to field conditions where temperatures ranged from -6.6°C to 35.8°C with a mean batch temperature of 9.7°C to 23.7°C. Latent period expressed as time from inoculation until the first visible symptoms ranged from 8 to 34 days. A shifted cumulative gamma distribution model with a base temperature of 0.5°C best described the relationship between number of lesions with pycnidia and accumulated thermal time. When defined as time to 50% of the maximal lesions with pycnidia, latent period was estimated as 297 and 313 degree-days above 0.5°C for USG 3209 and AGS 2000, respectively. The relationship between the inverse of latent period, defined as time to 50% maximal lesions with pycnidia, and mean temperature was best described using a linear model ($r^2 = 0.93$, $P < 0.001$). This study provides data that link wheat growth with SNB progress and will facilitate the construction of disease development models for use in timing of fungicide application.

Introduction

Stagonospora nodorum is the causal agent of *Stagonospora nodorum* blotch on winter wheat in wheat growing areas around the world (9). Leaf and glume blotches of wheat, especially SNB, cause small to moderate yield losses annually (4-6). The warm and humid conditions in the southeastern US provide an environment that is conducive to disease development. Higher plant densities, shorter rotations and increased nitrogen applications of wheat have resulted in increased disease pressure and this has necessitated the need for adequate

disease control options (7). There are many fungicides on the market that have very good to excellent activity against SNB including strobilurins, triazoles and strobilurin-triazole mixtures (8). Growers in the southeastern United States often apply fungicides on a prophylactic calendar basis even though they may not be necessary (7, 8). This is primarily due to the absence of a decision support system to aid growers in determining when and if it is profitable to apply fungicides. Wheat is a low-margin crop and helping growers reduce unprofitable fungicide application is an important contribution to improving profitability of this crop.

The relationship between *S. nodorum*, the wheat crop and the environment needs to be understood before control strategies can be improved upon. To establish models of disease development, the importance of environmental factors, such as temperature, must be analyzed in relation to the crop and the pathogen (2). The latent period, or the period between inoculation and sporulation, of *S. nodorum* was estimated by Shearer and Zadoks to be 6-36 days under moist environmental conditions, 10-49 days under alternate wet/dry conditions and 10-14 days at the optimum temperature of 21°C (3). However, the previous studies on the effect of temperature on latent period have been done in controlled or glasshouse environments and therefore, may not be comparable to field conditions. It has also been recognized that defining the latent period on a physical time scale may not always be accurate. There have been exceptional arguments in favor of using thermal time in accumulated degree-days when temperature is thought to play a role in disease development (1). Because temperature is hypothesized as a major factor in disease progression, we have sought to determine a latent period of *S. nodorum* that is measured in thermal time (degree-days). This information will help us to link crop growth models with disease progression. Our objective is to determine the effect of temperature on the latent period of *S. nodorum* on two cultivars of winter wheat under outdoor conditions.

Materials and Methods

Two cultivars of soft red winter wheat (*Triticum aestivum*) that are prevalent in southeastern US wheat germplasm and vary in resistance to *Stagonospora nodorum* were used: cv. AGS 2000, moderately susceptible; and cv. USG 3209, moderately resistant. These cultivars are inoculated at weekly intervals as seedlings, incubated under controlled conditions [Phytotron chamber, 21°C] and placed outside in a plot set up at Reedy Creek (Raleigh, NC). Ratings of disease symptoms are taken every 2-3 days by the same assessor.

Preparation of inoculum and plants. The *S. nodorum* isolate, designated NC-7-1, was isolated from a lesion on winter wheat debris collected from a field trial in Salisbury, North Carolina. A 0.2mL aliquot of spore suspension from NC-7-1 is subcultured onto V8 agar plates weekly and grown at 23°C under continuous black-light lamps in a growth chamber to promote sporulation. Inoculum is obtained by flooding the surface of a 10-day-old subculture with sterile distilled water, then scraping the agar surface using a sterilized glass rod to release spores. The spore suspension is then adjusted to 1×10^6 spores mL⁻¹, by dilution, using a haemocytometer. Seedling wheat plants are grown in 9 cm pots in a controlled environment chamber at 21°C until the emergence of the second leaf, and are then moved to a greenhouse (24°C). This allows the plants to acclimatize to outside weather conditions while limiting infections. Plants are maintained free from mildew by applying 50 mL of the pyrimidine fungicide ethirimol, as a soil drench, when needed. When four leaves are fully emerged, 8 pots of each cultivar (constitutes a batch) are thinned to two plants per pot. The second, third and fourth leaves are marked to aid in identification during disease assessment.

Inoculation procedure. Six pots out of each batch are used for inoculation and two are used as uninoculated controls. Planted pots are sprayed using a handheld spray bottle on a rotating platform; 75 mL spore suspension was used for each batch of six pots. Following spraying, each pot was enclosed in a separate polyethylene bag with distilled water in the base to maintain high humidity, and is then placed in a controlled environment chamber providing 12 h daylight and 21°C constant temperature to ensure consistent and successful infection of each batch. The bags are removed after 72 hours and the plants are placed in an outside plot at Reedy Creek exposed to ambient conditions. Temperatures are recorded hourly using a Watchdog data logger housed in a Gill radiation screen positioned above the potted plants.

Assessment of symptom development. Disease is recorded as the number of lesions with visible pycnidia of *S. nodorum* and is assessed every 2-3 days, from the time of inoculation until leaf senescence or values are constant. Each lesion with pycnidia is recorded with the help of a jeweler's lens (4.5X) on the tagged leaves (2-4) from the base of the plant.

Definition of latent period. Latent period generally represents the time from inoculation to the onset of symptoms. However, this definition may not always be the most accurate for describing the latent period. The definition that one uses for latent period is dependent on the types of relationships the pathogen has with the host. For this study, we define the latent period in three ways: time to first visible symptoms (LFVS), time to 50% maximum lesions with symptoms (L_{50}) and time to maximum observed symptoms (L_{100}). Symptoms in this study are defined as a distinct lesion with at least one pycnidium.

Data analysis. The ‘thermal time’ concept has been adapted by many in the field of horticulture to determine maturation times for crops. Essentially this concept states that stages of development of a biological system only occur when a certain number of heat units are accumulated and thus, link development to temperature. For data analysis, the thermal time per day was calculated using the formula, $TT = [(max\ Temp) + (min\ Temp)]/2 - T_{base}$. The thermal time was then accumulated over the period of exposure per batch. Two base temperatures (the minimal temperature at which the organism can continue development) were used. The accumulated thermal time was calculated above an assumed base temperature of $0^{\circ}C$. Thermal time was then accumulated over the optimized base temperature of $0.5^{\circ}C$. The data for maximum lesion number and lesions with pycnidia were pooled over pot, plant and leaves to obtain a mean for each batch. Using the analysis methods described in detail by Lovell (2), the increase in lesions with pycnidia post-inoculation, $N(t)$ was described using a shifted cumulative gamma distribution of the form:

$$N(t) = \begin{cases} 0, & TT(t) < p \\ N_{max} \int_0^{TT(t)-p} \frac{\alpha^{\beta}}{\Gamma(\beta)} u^{\beta-1} e^{-\alpha u} du, & TT(t) \geq p \end{cases}$$

where, N_{max} represents the maximum number of lesions that develops from a single inoculation event, $TT(t)$ is the accumulated thermal time above the optimized base temperature, α and β are estimable parameters of the gamma distribution, and p is the lag or estimated thermal time period from inoculation to the appearance of the first infinitesimally small symptom. The NLIN procedure in SAS (version 9.1, SAS Institute, Cary, NC) was used to optimize parameter estimates of the shifted cumulative gamma distribution function.

Results

From February 2009 to June 2009, 12 batches of plants were exposed to field conditions with temperatures ranging from -6.6°C to 35.8°C in an outdoor experiment at Reedy Creek. The mean batch temperatures for the period from inoculation to development of lesions containing pycnidia ranged from 9.7°C to 23.7°C . The maximum number of lesions per leaf varied between batches (2.39-13.45 for USG 3209 and 2.92-14.0 for AGS 2000). The time in days from inoculation to first visible symptom (LFVS) was determined for each batch and varied between 8 and 34 days. In figure 1, the latent period per batch, defined as time to first visible symptoms and measured in days, is plotted against the mean temperature per batch for cv. AGS 2000. All twelve batches are represented by the week of the year that they were exposed to field conditions. In general, the latent period decreases with increasing temperature showing a temporal dependence for development. The same trend can be seen with cv. USG 3209. The mean LFVS for cv. AGS 2000 (19.5 days) is slightly lower than cv. USG 3209 (21.3 days). LFVS for cv. AGS 2000 was shorter than cv. USG 3209 in seven batches and was never longer than cv. USG 3209. The greatest difference in LFVS between the two cultivars was five days. The time to maximum observed lesions with pycnidia, L_{100} , differed greatly between batches (27 days-54 days) and were temperature dependent with the longest time occurring at the coolest temperatures.

A linear regression analysis of the reciprocal of LFVS (1/days) against the mean temperature showed a positive correlation ($r^2 = 0.813$) (Figure 2). The temporal development of lesions with pycnidia is shown for five batches of cv. AGS 2000 that were selected because they represent the range of mean batch temperatures collected throughout the experiment and are inclusive of the two most extreme temperatures (9.7°C and 23.7°C) (Figure 3). When the time scale is physical and measured in days, the batches seem to differ in development. When placed on a thermal time scale (Figure 3b) using a base temperature of 0°C , disease progression appears to be much more similar. Similar results were found with cv. USG 3209 (Figure 4). The optimized base temperature for the model was estimated to be 0.5°C . The development of lesions with pycnidia for five representative batches of AGS 2000 and USG 3209 calculated above the optimized base temperature is depicted in Figure 3c and Figure 4c, respectively. The optimized parameters α , β and p for the shifted cumulative gamma distribution were not very different for AGS 2000 and USG 3209 (Table 1). Using the model, we were able to derive

estimates of the accumulated thermal time to 50% maximal lesions with pycnidia (L_{50}) and estimates of accumulated thermal time to 5% maximal lesions with pycnidia (L_5) (Table 2). A range in the length of latent period was calculated using these two estimates ($L_{50} - L_5$). Using extrapolation, the corresponding data in days was obtained. The relationship between time to 50% maximal lesions with pycnidia (days) and mean temperature was best described using a linear model ($r^2 = 0.928$, $P < 0.001$) (Figure 5).

Discussion

There have been very few studies of the effects temperature on the latent period of *S. nodorum* under field conditions. There have been many such studies done to determine the latent period under controlled environments. For example, in 1972 Shearer and Zadoks performed an experiment to describe the latent period, defined as time to first visible symptoms, of *S. nodorum* under constant postinoculation temperatures ranging from 5°C to 25°C and under various moisture levels (dry treatment, wet treatment and a mixture of wet and dry treatments). They reported latent period estimates of 6-36 days under wet treatments, 10-49 days under a mixture of treatments and no development of symptoms under the dry treatment (3). In our analysis of field data, we have determined a range in latent period (days to first visible symptoms) as 8-34 days. This is well within the range that was provided by the controlled environment experiment; however, our estimates provide a more narrow range.

Latent period estimates given by field observations in the past have been predicted by using an estimated date of spore arrival. In our experiment, all environmental variation (including spore arrival) during the germination process was minimized through providing uniform and optimal germination conditions among all batches. In the study conducted by Shearer and Zadoks, observation intervals are quite large, having only 5 observation periods (3). There are two problems with long periods between observations. First, when latent period is defined as the time to first visible symptoms, there is a loss of accuracy when observation times are spread out. Also, it has been shown here that higher temperatures lead to shorter latent periods and thus, sampling times are not sufficiently short enough to capture the true length leading to loss of accuracy. The use of the shifted cumulative gamma distribution provides a way to reduce the problems associated with short latent periods and long periods between observations (2).

The range of temperature captured in this experiment is consistent with temperatures found in growing regions of the southeastern US, including the state of North Carolina during February to June. The latent period of *S. nodorum* was found to be more accurately explained using a thermal time scale as opposed to a physical time scale. The estimation of the base temperature of 0.5°C, as opposed to the assumed base temperature of 0°C, was a significant finding. In a similar study performed in the UK on a closely related fungal plant pathogen *Mycosphaerella graminicola*, the base temperature was found to be -2.37°C (2). Although the two pathogens may be related, it is not surprising that *S. nodorum* has such a high base temperature when compared to *M. graminicola* considering one of the major differences between the two pathogens is the optimal temperature for growth and development (16°C for *M. graminicola* and 22°C for *S. nodorum*).

The development of a working model for disease development for the *S. nodorum*-wheat pathosystem is a large step in the right direction towards management of the disease. Defining the latent period in thermal time allows us to link the development of disease with crop-growth models. The more we understand the relationship between the pathogen, host and its environment, the more we can supplement our control strategies.

Table 1. Parameters and statistics for the cumulative gamma distribution model used to describe the increase in the number of lesions with pycnidia with accumulated thermal time following inoculation of batches of winter wheat with *Stagonospora nodorum*

Parameter	Cultivar ^a	
	AGS 2000	USG 3209
Residual mean (s^2)	4.93 [172]	3.03 [169]
α	1.79 (0.66)	2.06 (0.84)
β	85.01 (0.00)	73.85 (0.00)
p	249.40 (42.71)	255.60 (54.13)

^aAGS 2000 and USGS 3209 are susceptible and moderately resistant to *Stagonospora nodorum* blotch, respectively. Values in square brackets and parenthesis represent degrees of freedom and standard errors, respectively.

^b Parameter estimates for α (shape parameter), β (scale parameter) and p (lag parameter) are given in degree-days based on a base temperature of 0.5°C.

Table 2. Estimates of thermal time required to fulfill the latent period of *Stagonospora nodorum* blotch, defined as either time from inoculation to 5% of lesions with pycnidia (t_5), or 50% of lesions with pycnidia (t_{50}) and range in length of latent period for winter wheat cultivars AGS 2000 and USG 3209 inoculated by *Stagonospora nodorum*

Defined latent period	Cultivar	Thermal time (degree-days)	Standard error
5% maximum lesions (t_5)	AGS 2000	291.1	3.07
	USG 3209	280.3	2.55
50% maximum lesions (t_{50})	AGS 2000	313.7	5.41
	USG 3209	297.4	4.21
Range in length of latent period ($t_{50} - t_5$)	AGS 2000	22.6	...
	USG 3209	17.1	...

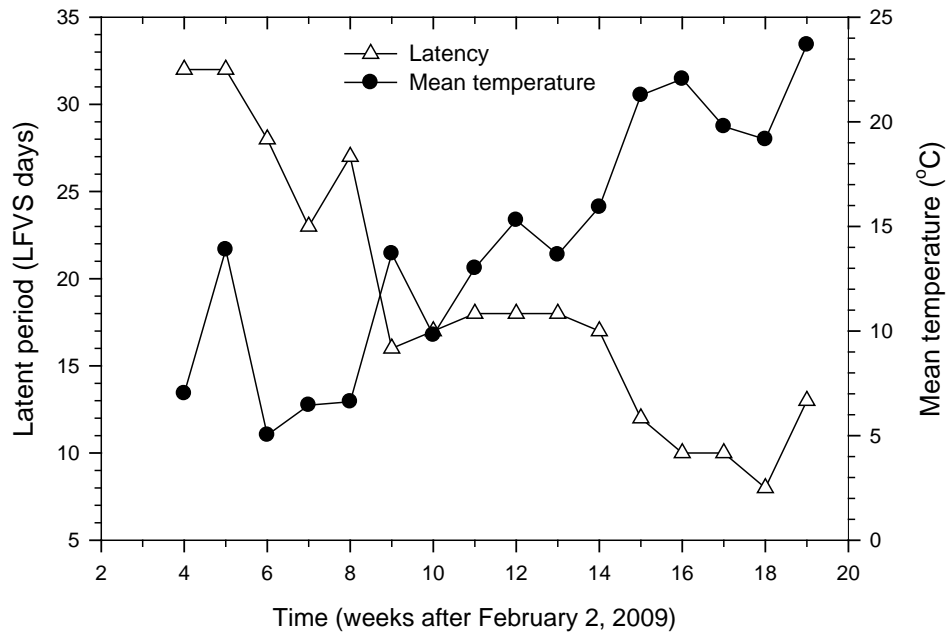


Figure 1. Latent period (days) from inoculation of *Stagonospora nodorum* pycnidiospores to first lesions with pycnidia and corresponding mean air temperature for 12 batches of winter wheat cv. AGS2000 exposed to ambient field conditions.

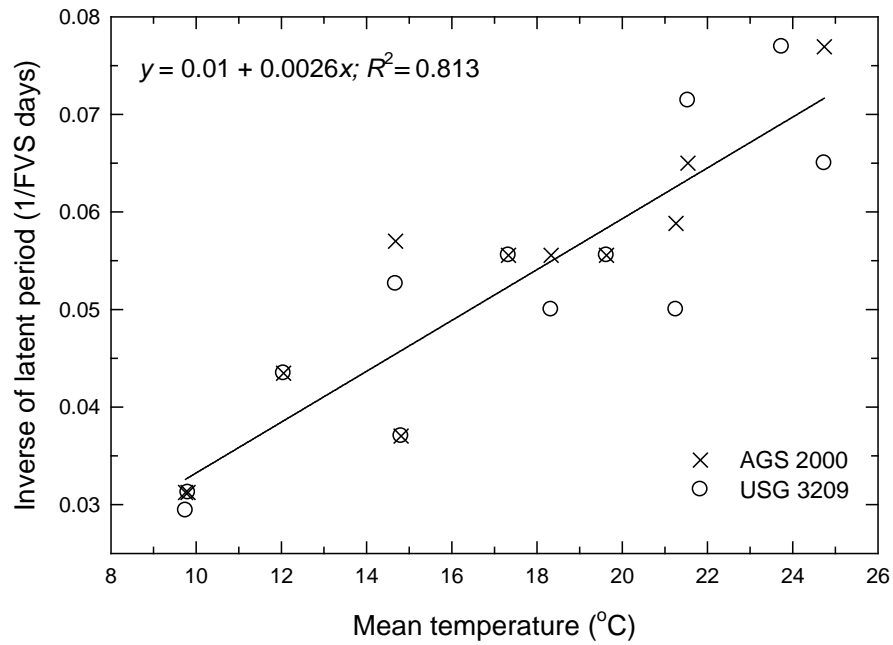


Fig. 2. Inverse of latent period (1/days) from inoculation of *Stagonospora nodorum* to observation of the first lesions with pycnidia for all batches of winter wheat cultivars AGS 2000 and USG 3209. The line depicts a fit of batch mean data to a linear model based on linear regression analysis.

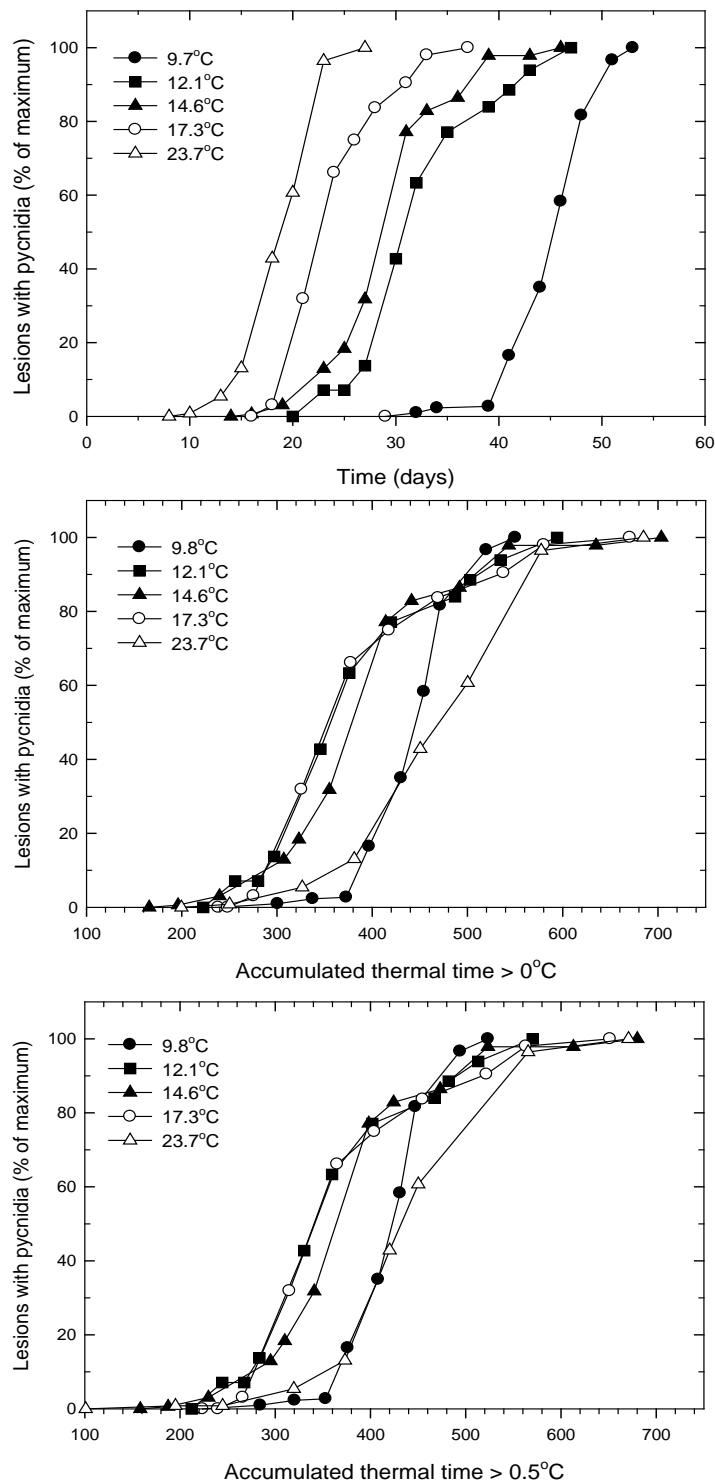


Fig. 3. Temporal increase in the number of lesions with pycnidia (as percentage of maximum number of observed lesions) for five batches of cv. AGS 2000. Data are presented based on three different time scales: **A)** days after exposure, **B)** accumulated thermal time above 0°C and **C)** accumulated thermal time above the optimum base temperature (0.5°C).

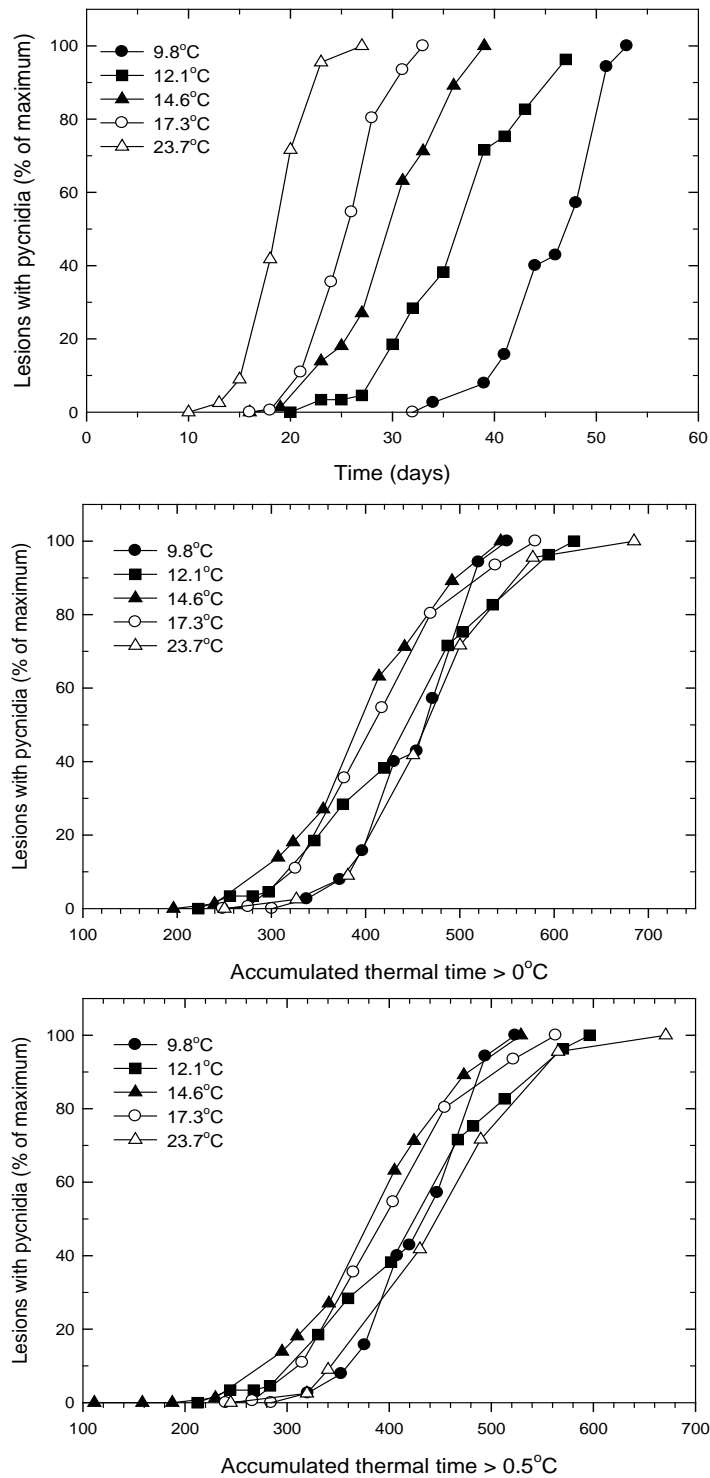


Fig. 4. Temporal increase in the number of lesions with pycnidia (as percentage of maximum number of observed lesions) for five batches of cv. USG 3209. Data are presented based on three different time scales: **A)** days after exposure, **B)** accumulated thermal time above 0°C and **C)** accumulated thermal time above the optimum base temperature (0.5°C).

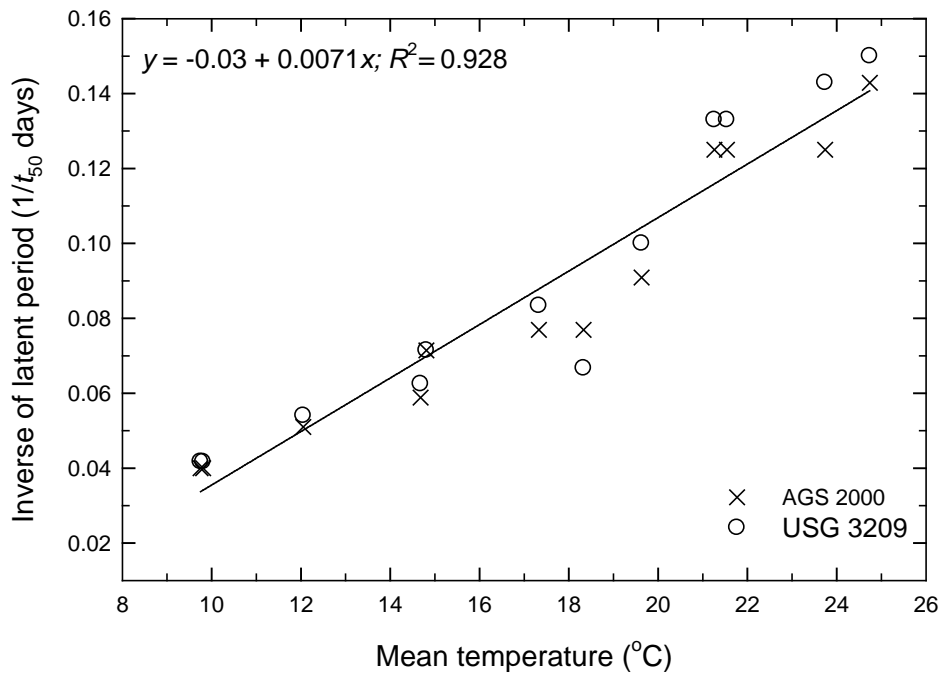


Fig. 5. Inverse of latent period (1/days) from inoculation of *Stagonospora nodorum* to 50% of the maximum number of lesions with pycnidia (t_{50}) for all batches of winter wheat cultivars AGS 2000 and USG 3209. The line depicts a fit of batch mean data to a linear model based on linear regression analysis.

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Projects Conducted in the Phytotron 2009

Air Quality

Booker, Fitz & Kent Burkey

Plant Responses to Ozone - Lab Demonstration for CS 714

Burkey, Kent & Fitz Booker

Physiological and Biochemical Responses to Ozone

Biological Science

Haddad, Nick & Linda Rudd

Tobacco Plant Lesson for Bio 260, Undergraduate Ecology Course

Crop Science

Brooks, Ashley

Identification of Allelopathic Rye Lines

Brown-Guedira, Gina and Mohammed Guedira

Analysis of Vernalization and Photoperiod Requirements of Winter Wheat

Carley, Danesha and Thomas Rufty

Evaluation of Temperature and N Regime on Cool-Season Turf Grass

Dewey, Ralph & Minesh Patel

Development of Transgenic Turf Varieties With Enhanced Agronomic Performance

Haigler, Candace and Bir Singh

Investigation of Novel Mechanisms of Cotton Fiber Development

Haigler, Candace and Bir Singh

Function & Regulation of Arabidopsis Tracheary Element Peptidase

Holland, Jim & Charlie Zila

Fine-Mapping A Photoperiod Response Gene in Maize

Kuraparthi, Vasu

Molecular Characterization of Wheat-Ae. Umbellulata Introgression Carrying Novel Rust Resistance Gene

Kuraparthi, Vasu & Ryan Andres

Germplasm Enhancement in Cotton Using Wild Related Species

Maloney, Peter

Maturity Study for Ril Pop

Milla-Lewis, Susana, Carolina Zuleta, Franco Villegas & Thomas G Isleib

Screening of Peanut Germplams for Tomato Spotted Wilt Virus (Tswv) Resistance.

Milla-Lewis, Susana, Lane Tredway & Maria Zuleta

Screening St. Augustine grass Germplasm for Gray Leafspot Resistance

Qu, Rongda

Agrobacterium-Mediated Transformation of Tall Fescue for Disease Resistance and Drought Tolerance

Qu, Rongda & Bingwu Wang

Elevation of Nicotine Level in Tobacco Through Genetically Engineering

Qu, Rongda & Niki Robertson

Drought and Salt Tolerance in Rice

Qu, Rongda & Ruyu Li

Switchgrass Transformation for Improved Ethanol Production

Qu, Rongda

Using Tap to Isolate Exon Junction Complex From Arabidopsis

Reynolds, Casey & Grady Miller

Athletic Field Paint Effects on Turfgrass Photosynthesis

Reynolds, Casey & Grady Miller

Athletic Field Paint Effects on Bermudagrass Photosynthesis

Riar, Mandeep & Thomas Rufty

Determining The Realized Niche of *C. Benghalensis* By Studying The Effects of Nutrients and Light

Richardson, Rob & Sarah True

Evaluation of Climate Effects on Giant Salvinia Response to Herbicides

Rufty, Thomas & Shannon Sermons

Effect of Growth Rate on Water Use By Tall Fescue

Schroeder-Moreno, Michelle & Dolly Watson

Effects of Temperature, N Deposition and Mycorrhizal Fungi on Switchgrass Growth & Nutrient Uptake

Seversike, Thomas & Thomas Rufty

Effects of Drought on Transpiration in Glycine Soja

Sinclair, Thomas, Thomas Seversike & Thomas Rufty

Response of Maize and Sorghum to Vapor Pressure Deficit

Sinclair, Thomas

Screen Soybean Genotypes for Transpiration Sensitivity to AgNO_3

Spears, Jan

Crop Science UG Class Labs

Spears, Jan

Crop Science Teaching - Light

Stiff, Michael, & David Danehower

Coq10 Production in Cultivated Tobacco Containing Atppt1

True, Sarah & Rob Richardson

Giant Salvinia and Water Hyacinth Control with Galleon and Sonar at Different Light and Temp Regimes

Entomology

Burrack, Hannah & Monique Rivera

Behavior and Management of Polyphagous Tobacco (*Nicotiana glauca*) Herbivores

Cardoza, Yasmin & Janet Griffiths

Elucidating Soil Factors Modulating Plant-Arthropod Interactions

Hunt, Jim

Nourishment and Caste in a Social Wasp, *Polistes metricus*

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Rose Transformation

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Expression of Archaeal Vactive Oxygen Detox Enzymes

Plant Biology

Boss, Wendy & Imara Perera

Regulation of Phosphoinositide Metabolism & Plasma Membrane Sig

Boston Rebecca & Jeff Gillikin

Characterizing Arabidopsis Mutants with Altered UPR Response

Hilton Jacob, Wade Wall & Thomas Wentworth

Determining Constraints to Establishment of *Pyxidantha brevifolia*

Hoffmann, William, Renee Marchin & Wade Wall

Role of Soil Pathogens in Determining Density Differences in Two Closely Related Tree Species

Hoffmann, William & Renee Marchin

Role of Soil Pathogens in Determining Density Differences in Two Closely Related Tree Species

Johnson, Marc

The Effects of Biotic and Abiotic Stressors Associated With Climate Change on Soybean (*Glycine max*)

Dalal-Kajla, Jyoti & Heike Sederoff

Functional Characterization of a Gravity-Regulated Sterol-Binding Protein in *Arabidopsis thaliana* Roots

Khodakovskaya, Mariya & Heike Sederoff

Developmental Changes Associated with Transgenic Modification of ER Calcium

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Development of a Vigs System for Rose

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Mapping a Geminivirus Resistance Gene

Rojas-Pierce, Marcela & Maria Fernanda Rodriguez-Welsh

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Xie, Deyu

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Balint-Kurti, Peter

Investigation of Effect of Fungal Extract on Plants

Balint-Kurti, Araby Belcher & Jose Santa Cruz Hidalgo

Reassociation of Defense Gene Expression with the Southern Corn Leaf Blight Resistance Locus Rhl1

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Natural Genetic Variation for Innate Immunity in Maize

Bartz, Faith, Marc Cubeta & Margo Daub

Rhizoctonia Solani Aggressiveness Bioassay on Tomato

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Association of Defense Gene Expression with the Southern Corn Leaf Blight Resistance Gene Rhl1

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Evaluation of the Soybean Gmubi-Promoter Activity during Nematode Infection

Adams, Mike

Epidemiology and Genetic Diversity of The Cucumber Downy Mildew, *Pseudoperonospora cubensis*

Hu, Shuijin & Cong Tu

Ecology and Management of Earthworms in Turfgrass Systems: Pesticide Impacts

Ma, Bangya

In Vivo Sensitivity of *Pythium volutum* to Fungicides

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The Relationship between Temperature and Symptom Expression in Pythium Root Dysfunction

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Biology and Management of Fairy Rings in Golf Putting Greens

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Effect of Temperature on Latent Period of *Septoria nodorum* Blotch on Winter Wheat

Ojiambo, Peter & L.Felipe Arauz

Infection Studies on Cantaloupe Downy Mildew

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Grafting Tomato for Induced Resistance

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Disease Assessment in A F2 Population of Maize for Southern Leaf Blight Resistance

Shew, Barbara & Joyce Hollowell

Carpogenic Germination of *Sclerotinia minor*

Upchurch, Greg

Detached Leaf Assay for Fungal Pathogens of Soybean

Upchurch Greg

Defense Gene Expression in Pathogen Inoculated Susceptible and Resistant Soybean Varieties

Upchurch, Greg

Effect of Temperature on Expression of Soybean Chloroplast Fatty Acid Desaturases Fad7 and Fad8

Parr, Mary & Julie Grossman

Nitrogen Fixation in Winter Annual Legumes

Seehaver, Sarah Julie Grossman

Rhizobia Diversity of CEFS Long Term Farming Systems Soil

Visiting Scientists

Mcnamara, Dawn

Syngenta Biotechnology

Niblett, Chuck

Assess Disease Resistance of Transgenic Plants

Weston Brigitte

BASF - Canola Project 01