Title: **Utilizing Diverse Strawberry Germplasm for Developing Genetic Improvement Strategies for the Southeastern United States**

Research Progress Report
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Objectives:

1. Evaluate the performance of regionally and/or nationally important strawberry cultivars from most of the major public breeding programs in the United States.
2. Evaluate native *Fragaria virginiana* and *F. chiloensis* germplasm for useful horticultural traits.
3. Assess the genetic relatedness of all the above germplasm using molecular markers.
4. Screen the germplasm collection for resistance to *Colletotrichum gloeosporioides*
    a. Added objective per reviewers comments

Justification:

Strawberry production in the United States (52,200 acres) is dispersed throughout the country and valued at 1.4 billion dollars (NASS, 2005). Major production regions include California and Florida, however, 49 states reported acreage in 2002 including Alaska and Hawaii (census of agriculture, 2002). The widespread cultivation of strawberries is due in part to the interspecific

nature of the commercial strawberry (*F.* x *ananassa* Duchesne). The chance hybridization occurred almost 300 years ago between two native American octoploid species, *F. chiloensis* Duchesne and *F. virginiana* Duchesne. The limited number of founder genotypes forming this hybrid population has lead to a severe bottleneck and is evident by the few cytoplasmic and nuclear contributors to the contemporary gene pool. Recently, strawberry geneticists have become increasingly interested in utilizing wild populations of the two progenitor species to increase the genetic base. An elite core collection of 38 accessions which contain *F. virginiana* and *F. chiloensis* has been identified for use in the genetic diversification and improvement of contemporary strawberry germplasm.

Breeding programs are active across the country including the Pacific Northwest, California, Wisconsin, Michigan, New York, Maryland, North Carolina and Florida and they continue to release new and improved genotypes. Within each program, industry requirements drive selection in sometimes different directions. For example, in the Pacific Northwest there has been a historic presence of a processing industry. Therefore, cultivars for this region have dark internal and external fruit color, high sugar and high acid levels. Conversely, the California industry has mostly been motivated by the fresh market traits which include adequate firmness for shipping, appearance and fruit size. Other programs such as New York, Maryland and North Carolina attempt to develop genotypes that are acceptable for direct market or pick your-own sales. Important traits for this market include flavor, appearance, firmness and size. In addition to the different market drivers of selection, cultural differences exist between the regional industries as well. Plasticulture has begun to replace matted row production in many regions of the country, however, matted row continues to be the predominant system in areas of the Northeast, mid-West and Pacific Northwest. Much of the material originating from these diverse programs has yet to be tested in the southeast and could prove useful as potential cultivars for production and/or useful sources of traits for some of our yield limiting pressures.

Methods:

An extensive strawberry cultivar trial was established at the Southern Piedmont AREC in Blackstone VA during the fall of 2007 and fruited in the spring of 2008. Source material was selected from seven breeding programs that represent five distinct strawberry growing regions in the United States that included: the Pacific Northwest (Washington and Oregon), California, New York, the Mid-Atlantic (Maryland and North Carolina) and Florida. Cultivars were selected from each region based on nursery sales and overall performance in their respective environments. A second trial was established that evaluated selected entries from the super core germplasm collection comprised of *Fragaria chiloensis* and *F. virginiana* clones. This was the second year for this trial and results for the 2006-07 and 2007-08 harvests will be presented. The entries were tested using conventional plasticulture and evaluated for yield, fruit size, and firmness. Additional fruit quality data (sugar content, pH, total acidity and total antioxidant activity) were also evaluated.

The experiments were set up as randomized complete block designs with four replications. Each plot consisted of ten plants for the cultivar trial and 4 plants for the germplasm trial and was harvested twice a week. For each harvest date, a sub-sample of 10 uniform berries was taken and frozen at -70^{o}C. Each week's harvest was combined to obtain a representative 20 berry

sample and clarified juice was prepared by maceration followed by centrifugation. Soluble solid content (SSC) was determined using a bench top refractometer and the total antioxidant activity (TAA) was measured using the ferric reducing antioxidant power (FRAP) method. The FRAP method was performed using a Dynex 96-well microplate reader scanning at 595nm. The working FRAP solution consisted of acetate buffer (300mmol/L), TPTZ (2,6,6-Tri(2-Pyridyl)-s-triazine) and $FeCl_3$. This working FRAP solution was added to the juice sample and ddH_2O in a ratio of 30:1:3 for a final volume of 136 µl per well. The samples were analyzed in triplicate and an average OD value was obtained after incubating at 37ºC for 60 minutes. A ferrous sulfate heptahydrate solution ranging from 8000, 6000, 4000, 2000 and 1000 µmol/L was used to generate the standard curve. A separate standard curve was done for each 96-well plate. Total antioxidant activity was expressed as uM $FeSO_47H_2O$ equivalence. Any sample with a coefficient of variation greater that 10% was reanalyzed.

Disease resistance screening for anthracnose crown rot (*Colletotrichum gloeosporioides*) was performed in the spring of 2008 with plug plants of commercial cultivars and *F. vesca* germplasm. Isolates of the fungus were collected from diseased strawberry plants in Virginia during the winter of 2007-08. One highly aggressive isolate was selected for disease screening. Whole plants were screened in the greenhouse and inoculated with either 1) 500ul of a spore suspension (1.5×10^6 cfu) applied directly to the top of the crown or 2) dipping a dissecting needle into a pellet of conidia and inoculating the base of the crown by inserting the needle approximately 2mm. Pots were arranged in a randomized complete block design with four replications. Following inoculation, the greenhouse was maintained at 25°C and plants kept under intermittent mist for 48 hours. Plants were evaluated after 30 days and scored for the crown reaction (0-5, 0= no rot 5 = severely rotted) and percent crown rot. A detached petiole assay was also tested using petioles from uninoculated greenhouse plants. Petioles of intermediate age and uniform size were selected and trimmed to 7mm. Petioles were inoculated by either 1) dipping the basal end of the petiole 5mm into a 1.5×10^6 spore suspension or 2) puncturing the middle of the petiole with a sterile needle dipped into a pellet of conidia. Individual inoculated petioles were placed into 9mm Petri plates with moist filter paper and wrapped with parafilm to maintain high humidity. All plates were placed in a growth chamber at 25°C with constant light and arranged in a randomized complete block design with four replications. Lesion expansion was measured daily for seven days and correlation analysis was done with the whole plant greenhouse results. The data presented in table 4 is the lesion length after seven days of incubation.

Results:

Objective 1

The fall of 2007 was extremely dry with above average temperatures for the months of September, October and November. No significant disease or pest issues were noted during the fall or winter. March and April (Bloom periods for Blackstone) were characterized by normal temperatures with above average rainfall and very few frost events. No overhead irrigation was required and all frost protection was successfully accomplished using 1.2 oz/yd^2 row covers. Harvest began on April 18 and ended on June 2 for a total of 14 harvest dates. Results are presented in tables 1 and 2.

Chandler was the highest yielding cultivar in the trial although Camino Real, Camarosa, Bish and Tillamook all produced very respectful season totals. Camino Real ranked first for fruit weight (26.5 g) and was only statistically matched by Albion (25.2g) and Tillamook (24.5g). Camarosa and Camino Real appear to have a similar fruiting pattern, however, the superior fruit size, good firmness and open plant architecture of Camino Real makes it an attractive option for the shipping market. Bish produced slightly less marketable yields and smaller average fruit weight compared to Chandler. Both had similar distribution of fruit across the season but Bish had a weaker finish. Tillamook and Puget Reliance were the two best performing Pacific Northwestern (PNW) cultivars. Tillamook had better overall performance with large fruit and low unmarketable yields. All of the PNW entries had extremely soft fruit which will severely limit their use for shipping. Tillamook had adequate firmness for PYO and ranked well for flavor in some informal taste tests (data not shown). Galletta, the recent release from Jim Ballington's program, was extremely impressive during the first week of May when approximately 70% of the total harvest was picked (15,300 lb/A). This cultivar may be able to fill any potential marketing gaps for shipping or PYO during the early-mid season that Camarosa and Sweet Charlie are unable to satisfy. The production trend toward the later part of the season was a steady decline and resulted in a relatively short season. Clancy, a recent release from Cornell University bred for matted row culture, was large fruited, firm and had the longest fruiting season of the New York entries. This cultivar would be well suited for shipping, however, its cropping pattern was delayed about 6 days from Camarosa and Camino Real but it competed well in the mid to late season.

Sweet Charlie has been and continues to be the preferred early season cultivar. Botrytis fruit rot was heavy on this cultivar early in the season and contributed substantially to the high % unmarketable yield (17.0%). Fruit size was large (21.2g) and the fruiting season was long (35 days) with excellent early season production. Overall, total marketable yields were low and the mid and late season yields were very poor. As long as early production is the goal (i.e. first 2 weeks of picking) Sweet Charlie is still a good fit but certainly on limited acreage. Festival had excellent performance in this year's trial and began fruiting about 3-5 days later than Sweet Charlie. Marketable yields exceeded 24,000 lb/A of firm, deep red fruit. Fruit size was less than Sweet Charlie but it had greater mid and late season production.

Late season production is typically characterized by a decrease in fruit size and pounds per acre picked. Summer-like temperatures (90's) can spike sometimes in mid to late May. But more typically, around the first week of June conditions quickly deteriorate and turn the season off. May of 2008 was characterized by slightly below average temperatures which kept quality high, however, the first ten days of June were extremely hot and averaged 93 ^{o}F with 6 days above 95^{o}F. This situation put an abrupt end to the season for all cultivars in the trial. Ovation, a newer release from the USDA in Maryland, was the top yielding late season entry that produced twice as much as Chandler during the last week of May (12,000lb/A vs 6,000lb/A). Its cropping pattern was exactly opposite that of Sweet Charlie with poor performance during the early and mid season but excelled late. Ovation is a "big" plant, more so than Camarosa, and made picking in the dense canopy difficult.

Table 1. 2008 strawberry cultivar trial harvest data from Blackstone, VA.

Cultivar	Origin	Marketable Yield[z] (lb/A)	Fruit Weight[z] (g)	Fruit Firmness[z] (g)	Unmarketable Yield [y,z] (%)	Harvest Season 5%	50%	95%	Harvest Season (Days)
Chandler	**CA**	**34,008 a**	**18.9 def**	**590 fgh**	**8.6 ef**	**4/29**	**5/12**	**5/27**	**27**
Camino Real	**CA**	**28,620 b**	**26.5 a**	**798 bc**	**9.7 ef**	**4/29**	**5/12**	**5/29**	**29**
Carmine	FL	28,048 bc	13.1 i	663 def	11.3 e	4/29	5/8	5/27	27
Camarosa	**CA**	**27,770 bc**	**20.1 de**	**890 ab**	**11.0 e**	**4/29**	**5/8**	**5/27**	**27**
Puget Reliance	WA	27,247 bc	18.4 efg	373 k	9.2 ef	5/1	5/15	5/27	22
Bish	**NC**	**26,977 bcd**	**15.0 hi**	**548 ghi**	**11.1 e**	**4/29**	**5/12**	**5/27**	**27**
Tillamook	**UDSA- OR**	**26,769 bcd**	**24.5 ab**	**470 ljk**	**5.9 f**	**5/1**	**5/19**	**5/29**	**24**
Festival	**FL**	**24,256 cde**	**16.1 gh**	**958 a**	**8.6 ef**	**4/25**	**5/1**	**5/19**	**23**
Winter Dawn	FL	23,251 def	15.1 hi	720 cde	19.6 b	4/23	5/1	5/15	21
Galletta	**NC**	**22,378 ef**	**18.0 efg**	**633 d-g**	**8.7 ef**	**4/29**	**5/1**	**5/15**	**15**
Jewel	New York	22,349 ef	14.5 hi	675 def	16.9 bc	5/8	5/22	5/29	21
Clancy	NY	21,598 efg	23.4 bc	675 def	10.6 e	5/1	5/15	5/29	24
Ovation	USDA- MD	20,324 fg	19.0 def	575 f-i	6.2 f	5/8	5/22	5/29	21
Totem	British Columbia	20,319 fg	14.6 hi	510 hij	10.9 e	5/8	5/19	5/29	21
Honeoye	New York	20,228 fg	16.8 fgh	525 g-i	9.1 ef	4/29	5/8	5/22	22
L'Amour	NY	20,054 fg	18.4 efg	733 cd	15.3 cd	4/29	5/8	5/22	22
Sweet Charlie	**FL**	**19,861 fg**	**21.2 cd**	**613 e-h**	**17.0 bc**	**4/23**	**5/1**	**5/29**	**35**
Allstar	USDA- MD	17,751 g	16.0 gh	625 d-g	16.4 bc	5/1	5/12	5/27	22
Albion	CA	13,997 h	25.2 ab	868 ab	12.4 de	4/29	5/15	6/2	33
Hood	USDA-OR	10,284 i	13.1 i	445 jk	25.2 a	5/8	5/15	5/27	19

[z] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

[y] Unmarketable yield includes small (<8 grams), deformed and rotten fruit

Table 2. Total antioxidant activity (TAA) and soluble solid content (SSC) of the cultivars tested in Blackstone, VA during the 2007-08 season.

Cultivar	TAA[z,y]	SSC[y]
Festival	6926.2 a	6.6 fg
Totem	6825.4 ab	7.7 b
Carmine	6823.3 ab	5.6 hi
Chandler	6654.1 abc	6.5 fg
Camino	6478.8 bcd	5.5 i
Albion	6390.9 cde	6.7 efg
Sweet Charlie	6341.8 cde	6.8 def
Puget Reliance	6267.3 cde	7.6 bc
Galletta	6261.9 cde	6.5 fg
Winter Dawn	6242.6 cde	5.4 i
Bish	6226.9 cde	6.8 def
Camarosa	6190.6 de	6.2 gh
L'Amour	6167.7 de	7.2 bcde
Tillamook	6107.4 def	7.4 bcd
Hood	6076.9 def	9.3 a
Clancy	6023.3 def	7.0 cdef
Honeoye	5928.6 efg	7.0 cdef
Allstar	5723 fgh	7.5 bc
Jewel	5600.3 gh	7.4 bcd
Ovation	5354.1 h	6.9 def

[z] TAA is expressed as uM $FeSO_4 7H_2O$ equivalence

[y] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

Objective 2

Several of the super core accessions did not flower during the 2007 season which included *F. chiloensis* ssp. *pacifica* (PIs 551445, 551459, 612489, 612490), *F. virginiana* ssp. *glauca* (PI 612501), *F. virginiana* ssp. *platypetala* (PI 552471) and *F virginiana* ssp *virginiana* (PI 612320). These genotypes were eliminated from the 2007-08 planting and several new genotypes were added (PIs 551735, 602568, 612324 and 612497). Data is presented in tables 3 and 4.

Accessions that performed well in 2007 also had good performance for 2008. Superior genotypes tested this year included PIs 551735 and 612324. 551735 was the largest fruited entry of the trial with an average berry weight of 5.8 g and individual king berries during the season weighed over 10g. Plant size was compact yet open which allowed for good light and air penetration into the canopy. It appeared to be relatively resistant to leaf spot diseases and had very few rotten berries (data not shown). However, overall productivity was moderate over the season (124.7g or 0.3 lb/plant). 612324 was the most productive entry and yielded 304.2g or 0.67lb/ plant.

The highest ranking accessions for SSC were 551736 (10.0), 612570 (9.8), 612486 (9.2), 236579 (9.1) and 612495 (8.7). Although these were the highest ranked, most genotypes had excellent performance with only three accessions having SSC values less than 7.0. TAA was less variable among the accessions and provided modest statistical separation. However, using all fruit harvest data (yield, fruit size and sugar and TAA) several superior genotypes can be identified. *F. virginiana* genotypes that would be useful to incorporate into a breeding program include: 612323,612324,612486 and 612569. *F chiloensis* genotypes that had good yield, fruit size and quality characteristics include: 551736, 551735 and 236579.

Table 3. Total fruit weight per plant and average berry size for the 2007 and 08 harvest seasons.

		Total Wt (g)		Avg. Berry Wt.(g)	
PI number	Taxon	2007[z]	2008[z]	2007[z]	2008[z]
612569	***F. virginiana***	**207.5 a**	**176.2 b**	**2.2 c**	**2.0 cde**
551736	***F. chiloensis***	**180.0 a**	**103.7 de**	**4.0 a**	**3.0 b**
551735	***F. chiloensis***	**-**	**124.7 cd**	**-**	**5.8 a**
236579	***F. chiloensis***	**178.5 a**	**153.7 bc**	**3.2 b**	**2.7 b**
612323	***F. virginiana***	**157.7 a**	**283.0 a**	**1.9 cd**	**2.0 cd**
612324	***F. virginiana***	**-**	**304.2 a**	**-**	**2.1 cd**
612495	*F. virginiana*	98.9 b	33.1 fg	1.4 def	1.3 fg
551453	*F. chiloensis*	70.0 bc	-	0.6 f	-
612487	*F. chiloensis*	55.2 bcd	18.3 g	1.6 cde	1.5 ef
612486	***F. virginiana***	**51.2 bcd**	**125.6 cd**	**0.9 ef**	**2.0 cde**
612499	*F. virginiana*	38.4 cd	-	1.1 def	-
612488	*F. chiloensis*	25.6 cd	21.2 g	1.5 cde	1.2 g
612493	*F. virginiana*	24.5 cd	8.2 g	0.8 ef	1.0 gh
612492	*F. virginiana*	14.6 cd	5.2 g	0.6 f	0.8 h
612570	*F. virginiana*	9.9 d	18.2 g	1.4 ef	1.7 de
612497	*F. virginiana*	-	65.1 ef	-	1.6 ef
602568	*F. virginiana*	-	6.5 g	-	2.2 c

[z] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

Table 4. Total antioxidant activity (TAA) and sugar content (SSC) for the 2008 harvest of the super core accessions.

PI number	TAA[z,y]	SSC[y]
612495	6196.9 a	8.7 abc
612497	6074.7 ab	6.9 de
236579	**6056.9 abc**	**9.1 ab**
612492	5873.8 abc	4.6 f
612493	5869.4 abc	6.6 e
612486	**5858.8 abc**	**9.2 ab**
612569	**5541.9 abc**	**8.2 bcd**
612570	5538.8 abc	9.8 a
612323	**5519.6 abc**	**8.0 bcde**

551736	**5417.6 abc**	**10.0 a**
551735	**5402.3 abc**	**8.0 bcde**
612487	5375.7 bc	7.6 cde
612488	5267.2 bc	7.9 bcde
612324	**5237.2 c**	**7.8 bcde**

[z] TAA is expressed as uM $FeSO_4 7H_2O$ equivalence

[y] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

Objective 3

Molecular markers, simple sequence repeat markers (SSRs), were used in 2008 to assess the molecular genetic relatedness of the cultivated and wild germplasm. SSR markers were selected from published literature that showed cross amplification between *F. virginiana*, *F. chiloensis* and *F. x ananassa*. Amplified products were separated using a Li-Cor 4300 DNA analyzer. With addition of Dr. Allan Brown to the Plants for Human Health Institute, who specializes in molecular genetic diversity, we will be assessing the data quality and move forward with the appropriate analysis to describe the germplasm relationship.

Objective 4

Several commercially available cultivars that originate from breeding programs across the United States were evaluated for resistance to anthracnose crown rot (*Colletotrichum gloeosporioides*). Main effects of genotype and inoculation method were significant but not their interaction for the greenhouse and petiole assays. There was an increase in rate of symptom development and final disease severity for the needle inoculations vs. the spore suspension techniques. Therefore, the data in tables 5 and 6 are the mean scores for both inoculation methods combined. The whole plant greenhouse assay showed that most of the cultivars tested were highly to moderately susceptible (Table 5). The Florida cultivars Sweet Charlie and Winter Dawn were the most resistant genotypes. These two cultivars are planted on very limited acreage in the Mid-Atlantic where this disease is occurring more frequently.

The growth chamber petiole assay used to determine the response of strawberry cultivars to anthracnose crown rot was a simple and quick assay that unfortunately did not provide good correlations to the greenhouse reaction (Table 7). This test was repeated twice and will not be used in the future for assaying the plant's response to crown rot.

The results from the *F. vesca* screen showed that very little resistance is found within the accessions tested (Table 6). Future efforts could be directed at sampling more genotypes to identify a highly resistant diploid strawberry for use in studying the genetics of resistance in a less complicated genetic system.

Table 5. Crown rot reaction of cultivated strawberries incited by *Colletotrichum gloeosporioides* in the greenhouse and petiole lesion length from growth chamber assays.

Cultivar	Crown Score (0-5)[z]	Crown Rot %[z]	Lesion length (mm)[z]
Camino Real	4.0 a	69.4 a	36.0 ab
Tillamook	3.8 ab	61.3 ab	37.9 a
Allstar	3.6 ab	57.0 abc	38.5 a
Hood	3.6 ab	55.0 abc	33.9 abc
Totem	3.5 ab	53.1 abc	-
Albion	3.3 abc	50.6 abc	34.0 abc
Festival	3.2 abc	47.5 abc	35.8 ab
Jewel	3.1 abc	47.5 abc	38.0 a
Gaviota	3.0 abc	42.5 bc	36.4 ab
Bish	2.8 bc	43.1 bc	35.1 ab
Chandler	2.6 bc	40.0 bc	33.4 abc
Camarosa	2.3 c	34.4 c	31.3 bc
Sweet Charlie	**0.9 d**	**7.3 d**	29.4 c
Winter Dawn	**0.8 d**	**7.6 d**	34.3 abc

[z] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

Table 6. Crown rot reaction of *Fragaria vesca* germplasm inoculated with *Colletotrichum gloeosporioides* in the greenhouse.

Taxon	PI	Crown Score (0-5)[z]	Crown Rot %[z]
F. vesca ssp. *Californica*	551749	4.3 a	72.9 a
"	551513	3.9 a	49.1 bc
F. vesca ssp. *Americana*	552287	3.8 a	52.8 ab
F. vesca ssp. *Vesca*	551890	3.7 ab	45.5 bc
F. vesca ssp. *Bracteata*	551514	3.4 abc	40.3 bc
F. vesca ssp. *Vesca*	551498	3.3 abc	38.4 bc
"	551909	3.3 abc	37.7 bc
"	551908	3.3 abc	30.4 bc
F. vesca ssp. *americana*	551881	2.6 bc	36.9 bc
F. vesca f. alba	551841	2.4 c	27.1 c

[z] Means within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test P=0.05)

Table 7. Correlation coefficients for petiole lesion length (mm) of growth chamber assay inoculations and greenhouse assessment criteria (crown score and percent crown rot) on the commercial cultivars tested for resistance to anthracnose crown rot.

Evaluation Parameter	Petiole lesion length	Crown score	Percent crown rot
Petiole lesion length	1.00		
Crown score	0.39	1.00	
Percent crown rot	0.38	0.94	1.00

Conclusions and Impact Statement:

Maintaining adequate genetic diversity in breeding populations is extremely important for continuing to realize significant genetic gain from selection. However, identifying superior genotypes to be used for injecting such diversity needs to be a calculated endeavor. Utilizing wild accessions to accomplish such a goal will ultimately result in a temporary decrease in superiority of the population. Therefore, our work in characterizing the yield, fruit quality and disease resistance traits of a very diverse collection of cultivated and wild will help breeders to strategically select genotypes that will not only achieve diversity but also introduce novel or key agricultural traits.

The level of resistance to anthracnose crown rot (Colletotrichum gloeosporioides) identified in the commercial germplasm was disappointing. The incidence of this disease has been increasing and represents a serious threat to the southeastern industry. Therefore, it is paramount that efforts continue to develop new cultivars that have adequate field resistance to anthracnose and are highly productive.