THE DEVELOPMENT OF THE MODERN STRAWBERRY (*FRAGARIA X ANANASSA*): PHYSIOLOGY, BIOCHEMISTRY AND MORPHOLOGY OF PROGENITOR SPECIES (*F. VIRGINIANA* AND *F. CHILOENSIS*) AND RESULTING CULTIVARS

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THE DEVELOPMENT OF THE MODERN STRAWBERRY (*FRAGARIA X ANANASSA*): PHYSIOLOGY, BIOCHEMISTRY AND MORPHOLOGY OF PROGENITOR SPECIES (*F. VIRGINIANA* AND *F. CHILOENSIS*) AND RESULTING CULTIVARS.

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The cultivated strawberry (*Fragaria x ananassa*) in the northeastern United States has seen limited improvement in yield over the past century. Narrow germplasm diversity has been suggested to be a possible limitation and has resulted in evaluation of the progenitor species: *F. chiloensis* and *F. virginiana* for desirable characteristics. One of the possible avenues explored to improve the productivity of the cultivated strawberry is to increase the photosynthetic capacity through the use of *F. chiloensis* which has a high photosynthetic capacity. Previous research has relied exclusively on gas exchange data to quantify photosynthetic capacity; however, due to the complexity of regulatory processes that influence gas exchange it is difficult to identify the basis of the different gas exchange rates observed. Evaluation of the strawberry cultivar 'Jewel' and the two progenitor species was conducted to determine the basis of the observed differences in photosynthetic capacity. Results of this research suggest that the high photosynthetic capacity of *F. chiloensis* may be a based on higher light use efficiency, determined by chlorophyll fluorescence, and increased activity of key enzymes of the Calvin Cycle. As these photosynthetic parameters are based on gene expression, these higher rates may be genetically heritable.

In order to determine the most effective use of the wild species to improve the cultivated strawberry, a field study of cultivars released during the last century was

established to evaluate changes in horticultural and physiological characteristics that have occurred and to identify possible limitations to productivity. Results indicated that limited changes have occurred in carbon allocation patterns and photosynthetic characteristics and suggest that there may be opportunity to increase the productivity through altering carbon allocation patterns and increasing fruit number. The lack of change in photosynthesis suggests that improving photosynthetic capacity of the cultivated strawberry may lead to increased productivity. Further research evaluating hybrids of *F. chiloensis* and the cultivated strawberry may allow us to better exploit this characteristic.

BIOGRAPHICAL SKETCH

I grew up in beautiful British Columbia surrounded by mountains, ocean and berries. I started my plant science degree at UBC and then transferred to the Department of Plant Science at the University of Guelph where I completed my undergraduate degree and got turned on to the wonderful world of fruit by my advisor, Dr. Al Sullivan with whom I completed my masters degree. In 2004 I moved to Ithaca to start my PhD in berry crops with Dr. Marvin Pritts. I will be continuing my academic pursuits in the fruit realm in the Department of Horticulture at the University of Wisconsin-Madison.

ACKNOWLEDGMENTS

When I started my academic career I had no idea how many people would invest their time and efforts into my pursuit; and in retrospect I am amazed at the extent to which people have gone to make this experience as rewarding as it has been. I am extremely grateful for the opportunity to have had Marvin Pritts, as my advisor. He has been a mentor to me in matters of science, teaching, community service and has become a good friend. He will always be my benchmark of what a great horticulturist is. Despite having no experience in biochemistry when starting this degree, Lailiang Cheng was willing to take me on as a student and invest the time to develop my skills in biochemistry. I would also like to thank Tom Owens, an excellent teacher who always had an open door and Arthur Wilson, my guide through the fascinating world of education!

Such an endeavor would not be possible without the support of great friends and family. The friendships I have made during my time at Cornell have made this experience rich and I know I have gained friends for life. Despite the great distance, my family has been a constant source of encouragement and I have appreciated all of their visits, pep talks and prayers! I can confidently say that this degree would never have been completed without my best friend and greatest supporter, Matt, who has poured so much of himself into this effort. He has allowed me to do things I would never have done on my own and kept me sane and laughing the whole way through. And of course my little Sofia who came along for the last leg of the race with me and has made the finish line sweeter than I could have ever imagined it would be!

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CHAPTER 1

The Relationship Between Leaf N Status and Photosynthetic Characteristics of *Fragaria x ananassa* 'Jewel'.

Introduction

Nitrogen is a mineral nutrient that can act as a constituent of organic structures, osmoregulator or as a signaling molecule (Marschner 2002). The N status of the plant influences all levels of regulation in the plant from gene expression to gross morphological characteristics and is closely involved in carbon assimilation ($A_{CO2,}$ $_{\mu}$mol CO_2 $m^{-2}{\cdot}s^{-1}$). The relationship between N content in leaves and the affect on A_{CO2} can be evaluated based on the direct effect of N on photosynthesis as a constituent of photosynthetic proteins, as a regulator of photosynthetic enzymes or by the indirect effects through its impact on plant growth.

The relationship between N status and plant growth are well understood under low N conditions; however, under high N conditions, interactions with C assimilation and the plant's ability to store N in vacuoles have made the effects more difficult to understand. The generalized growth response to N is linear at low N levels, eventually reaching an asymptote (Lawlor 2002). This has been demonstrated in apples (Cheng et al. 2002) and in strawberry plants where, in addition, the response to N is dependent on the timing of application. Increased responses were observed with fall applications compared to spring (Acuna-Maldonado and Pritts 2008). N status also influences the carbon partitioning in the plant by lowering the root:shoot ratio at high N application rates (Agren and Ingestad 1987; Levin et al. 1989).

The response of plant growth and partitioning to N has an indirect effect on A_{CO2} by altering the canopy density and architecture, and the source:sink ratio in the plant. Increased leaf area will lead to increased light interception which, in turn, could allow for higher A_{CO2}. The benefit of increased leaf area however, is possibly negated by increased maintenance respiration and by the mutual shading that occurs in an increasingly dense canopy (Marschner 2002; Peri et al. 2003; Robson and Parsons 1978). These changes can also alter the sink capacity of the plant which regulates A_{CO2} through feedback mechanisms. The primary end products of photosynthesis are sucrose and starch and the concentration of these end products plays a key role in regulation of photosynthesis. If sucrose begins to accumulate, sucrose phosphate synthase (SPS) is deactivated leading to reduced amounts of inorganic phosphate (Pi) being recycled to the chloroplast. Pi is required for the export of triose phosphates from the cytoplasm through the there is increased storage of starch in the chloroplast (Stitt et al. 1990).

The N response of plants is also impacted by leaf structure and composition. With increasing N content, there is an increase in chlorophyll content (Evans 1983; Peri et al. 2002) which may increases the capacity to harvest light energy if optical properties are not already optimized. Increased length, width and leaf area along with decreased leaf thickness are typical N-induced changes in leaf morphology (Yoshida et al. 1969). Leaves with thicker, denser leaves (higher SLW) tend to have lower rates of A_{CO2}/unit N due to increased intraleaf shading or slower rate of diffusion of CO_2 to the carboxylation site (Reich et al. 1998a) which then leads to reduced rates of the biochemical reactions.

Nitrogen also directly affects photosynthesis as it is an essential constituent of key proteins involved in photosynthesis. Rubisco is the most abundant protein in the leaf, accounting for up 20-25% of total leaf proteins (Evans 1989) and is considered to be the limiting factor to photosynthesis under light saturating conditions and under low

CO_2 conditions (Stitt and Schultze 1994). The activation state of Rubisco was also found to decrease linearly with increasing leaf N in apples (Cheng and Fuchigami 2000). In N deficient apple trees, several other key enzymes involved in the regeneration of ribulose 1,6-bisphosphate (RuBP) were shown to be limiting to photosynthesis (Chen and Cheng 2004).

There are several tools that can be used to evaluate potential limitations to photosynthesis, including chlorophyll fluorescence, gas exchange, growth analysis, measurement of enzyme activity and carbohydrate status. The objective of this study was to determine physiological or biochemical bases for the response of strawberry plants to N.

Materials and Methods

Plant Material. One year old dormant runners of *F. x ananssa* 'Jewel' were obtained from propagators and planted 12 May 2005 into 4.4 liter pots filled with 1:1 mix of sand:Metro mix® (Scott's Co., Maryville, Ohio). The plants were grown under natural conditions at the Cornell Orchards in Ithaca, New York, USA (Lat. 42.44 N, Long.76.50 W).

Treatments. All plants were watered with unfertilized water for three weeks plus two applications of Hogland's solution with 10mM nitrogen. Three weeks after planting, treatments of a modified Hoagland Solution (Cheng and Fuchigami, 2000) with an N (NH_4NO_3) concentration of 0, 5, 10, 15 or 20 mM were applied twice a week for the duration of the experiment. The experiment was designed as a randomized complete block experiment with 5 blocks and 5 plants per treatment in each block.

Gas Exchange Data. Gas exchange data were collected using the Li-6400 (Li-Cor Inc., Lincoln, Neb.) infrared gas analyzer. Conditions were constant for all

measurements; ambient CO_2 (360μmol mol^{-1}), photosynthetic photon flux density (PPFD) of 1300 μmol $m^{-2}s^{-1}$, temperature was maintained at 25°C ± 1°C. Light adapted measurements of CO_2 assimilation (A_{CO2}) were made once a week on a recently expanded leaf between 10:00-14:00. Light response curves with light levels ranging from 0 to 2000 μmol m^{-2} s^{-1} PFD and A_{CO2}-C_i curves with CO_2 concentrations ranging from 45ppm to 1500 ppm were generated on a recently expanded leaf of three plants per treatment and data for each treatment were pooled. Dark respiration measurements were made on a recently expanded leaf during predawn hours after a seven hour dark period.

Chlorophyll Fluorescence. Chlorophyll fluorescence was measured using the Li-6400 infrared gas analyzer with the chlorophyll fluorescence head attached to allow simultaneous gas exchange and fluorescence measurements. Measurements were made under full sunlight at noon to attain data of the fluorescence yield of leaves adapted to ambient light conditions (F) and the maximum light-adapted fluorescence yield when exposed to a saturating pulse of light (F_m'). These variables were then used to calculate the effective quantum yield of PSII ($\Phi PSII=F_m'-F/F_m'$). Predawn measurements were made on dark-adapted leaves to determine Fv/Fm. Fluorescence data were collected weekly throughout the experiment.

Calvin Cycle Enzymes. One cm^2 leaf disks were collected from three plants in each experimental unit at noon under full light (PFD 1700μmol $m^{-2}s^{-1}$) and immediately placed in liquid N_2. Material was stored at -80°C until the time of the assay. Rubisco, NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC1.2.1.12), fructose-1,6-phosphotase (FBPase, EC3.1.3.11), and sucrose phosphate synthase (SPS, EC2.4.1.14) were extracted according to protocol described by Chen and Cheng (2003). Rubisco, GAPDH, FBPase and SPS extractions were carried out using a total of three leaf disks. In a precooled mortar and pestle, the leaves were ground in

1.5mL of a buffer solution containing 50 mM Hepes-KOH, 10mM $MgCl_2$, 2mM ethylenediaminetetraacetic acid (EDTA), 10mM DTT, 1%(v/v) Triton X-100, 5% (w/v) bovine serum albumin (BSA), 10% (v/v) glycerol, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged for 5 min at 13,000rpm and the supernatant was used immediately for spectrophotometric analysis of enzyme activity (Chen and Cheng.

Non-structural Carbohydrates. Starch, glucose, sucrose and fructose were measured from leaf discs that were sampled at dusk, after a 15-hour light period and again from the same leaf at pre-dawn after approximately 8 hours of dark. Leaf discs were punched with a one cm^2 punch and immediately placed in liquid N_2 and stored at -80^{o}C until analysis. Starch measurement was carried out according to Chen and Cheng (2003), extraction was carried out by grinding three frozen leaf discs in four ml 80% ethanol, placing homogenate in a 80^{o}C water bath for 30 mins and then centrifuging at 13,000 rpm for five mins. Tissue was re-extracted two more times and supernatants were combined. After supernatant was removed, the remaining pellet was boiled in two mL of 0.2 M KOH for 30 mins then the pH was adjusted to 4.5 with 1M acetic acid. Digestion of starch was then carried out by adding 50 units of amyloglucosidase (EC3.2.1.3) at 55^{o}C for 30 min, then stopping the reaction by placing in boiling water for one minute, cooling and diluting to 5 mL with H_2O_2 and centrifuging for 5 mins. Glucose in the supernatant was measured spectrophotometrically at 340nm (Jones, 1977) using a coupled enzyme assay.

Soluble sugars were determined using a GC-quadrupole/MS. Extraction was carried out on approximately 100mg of frozen leaf tissue ground in a pre-cooled mortar and pestle, enzymatic activity was quenched by adding 1400µl 100% MeOH, and 60µl Ribitol was added as an internal standard. Sample was placed in a thermomixer at 70^{o}C at 950rpm for 15 mins, then centrifuged for 10 mins at 11000rpm. Supernatant was

transferred to a glass vial and 750 µl $CHCl_3$ and 1500 µl dH_2O was added and mixture was vortexed, then centrifuged for 15 mins at 22000rpm. The polar phase was then dried in a vacuum concentrator, sample tubes were filled with argon gas and stored at -80°C. To derivatize, dried samples were resuspended in methoxyaminhydrochloride and shaken (950rpm) for two hours in a thermomixer at 37°C . MSTFA was then added to the samples and shaken (950 rpm) for an additional 30mins at 37°C. 1 µl of the derivatized sample was injected into the GC at 230°C with a helium gas carrier (1mL/min). Chromatography was performed using a 30mm RTX-5Sil MS capillary column (0.25mm diameter, 0.25 µm film thickness) with a 10 mm integrated guard column.

Leaf Chlorophyll. Ten ($1cm^2$) leaf disks were extracted in 10mL acetone overnight in the dark and then centrifuged. Absorbance of the solution was measured at both 664nm and 647nm. Calculations were then carried out using previously published extinction coefficients (Graan and Ort 1984).

Growth Analysis. At the end of the trial, plants were destructively harvested. Fresh and dry weights were recorded for all components.

Results

Growth Analysis. Trends were the same for fresh and dry weights, so only dry weight data are shown. There was a significant increase in all components (leaf, runner, crown and root dry weight) with increasing N (Figure 1-1). Shoot components (leaf and runner) showed a linear response to increasing N, whereas root and crown growth had a more curvilinear response (Figure 1-1). Leaves responded to increasing N by increasing in size and number (Figure 1-2); there were not any significant changes in specific leaf weight across treatments. There was no significant difference observed in yield between treatments.

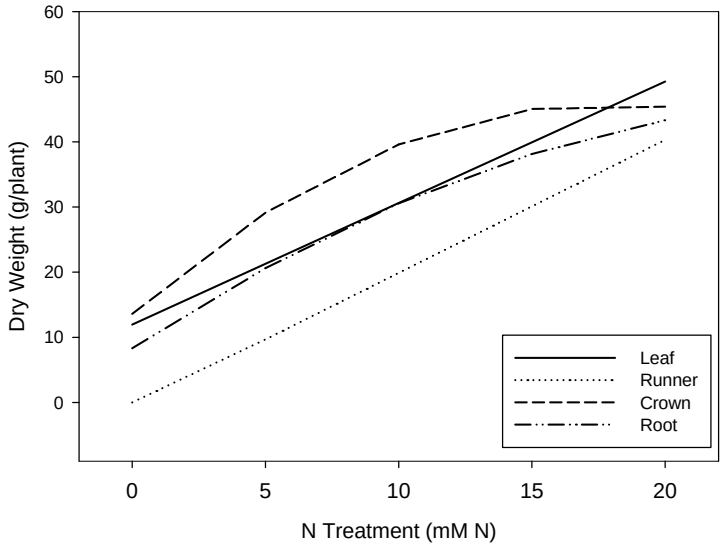

Figure 1-1 Growth response of 'Jewel' strawberry plants grown under 5 different N levels. Growth analysis conducted in September 2007.
Regression Equations: Leaf= 11.96 + 1.86*N (R^2=0.81, P<.0001); Runner = -0.55 + 2.04*Trt (R^2=0.67, P<.0001); Crown = 23.70 + 1.59*Trt - 0.10*$(Trt-10)^2$ (R^2=0.32, P<.0001); Root= 12.73 + 1.78*Trt - 0.047*$(Trt-9.65)^2$ (R^2=52, P<.0001).

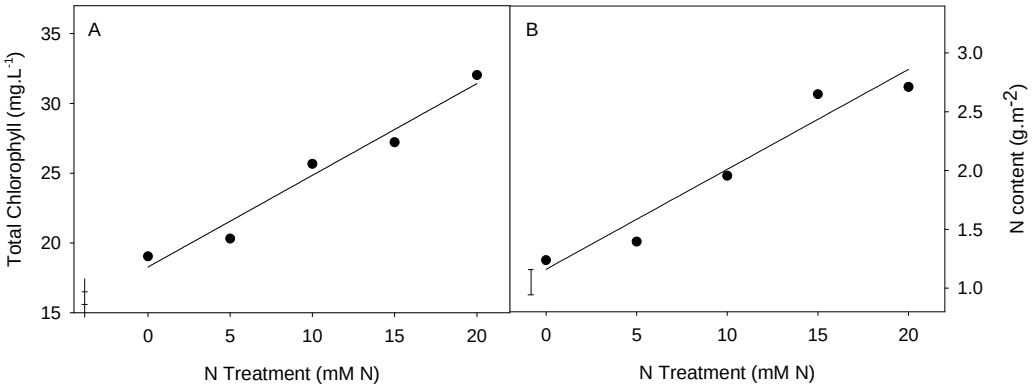

Figure 1-2. Response of leaf size (A) and leaf area to N treatment.
Regression Equations: (A) y=45.59+ 3.05*N - 0.11*$(N-9.83)^2$ (R^2=0.56, P<.0001); (B) y = 792.56 + 156.10*N (R^2=0.75, P<.0001).

Chlorophyll and Nitrogen Content. Both chlorophyll content and leaf N content increased linearly with the increasing N treatments (Figure 1-3).

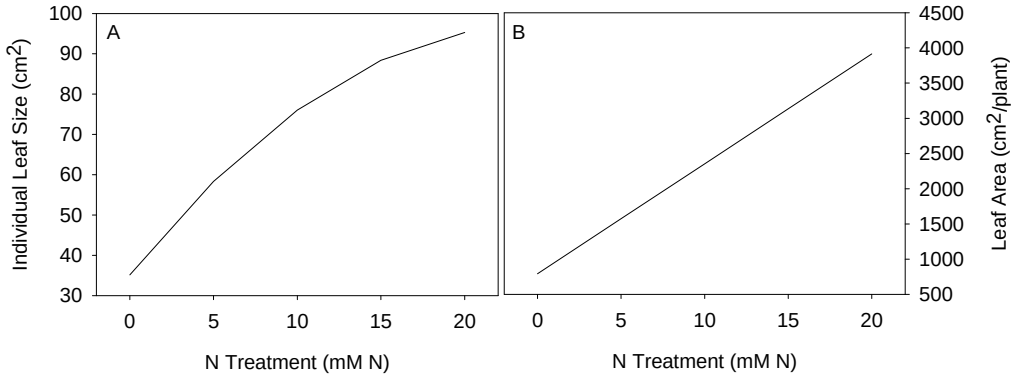

Figure 1-3 Effect of applied N treatment on chlorophyll content (A) and leaf N content (B) of potted 'Jewel' strawberry plants. Each point represents the mean (± standard error) of 4 replicates.
Regression equations: (A) y=18.28 + 0.66x (R^2=0.98, P=0.0029), (B) y=1.16+0.085 (R^2=0.94, P=0.016).

Gas Exchange and Chlorophyll Fluorescence. There was a significant increase in pre-dawn dark respiration rates with N treatments (Figure 1-4A). N status in the plant had a significant effect on the Fv/Fm values until the 10mM treatment after which additional N did not have any impact (Figure 1-4B).

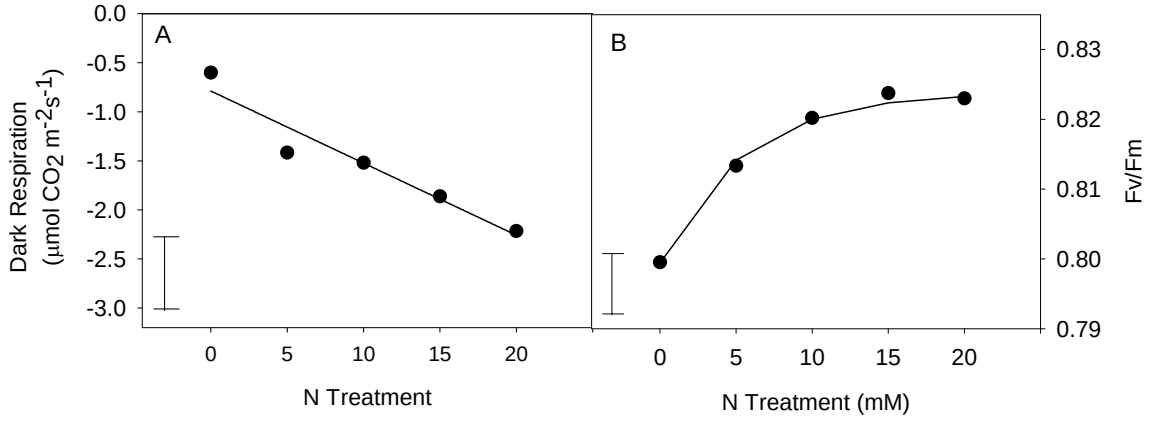

Figure 1-4. Response of dark respiration rate (A) and Fv/Fm (B) to different N treatments of 'Jewel' strawberry plants treated with different levels of N fertilizer. Regression Equations; (A), y= 18.28+0.66x (R^2=0.98, P0.0029), (B) =0.82-0.025$^{(-0.1841x)}$ (R^2=0.0058, P=0.0007). Standard error indicated by error bar.

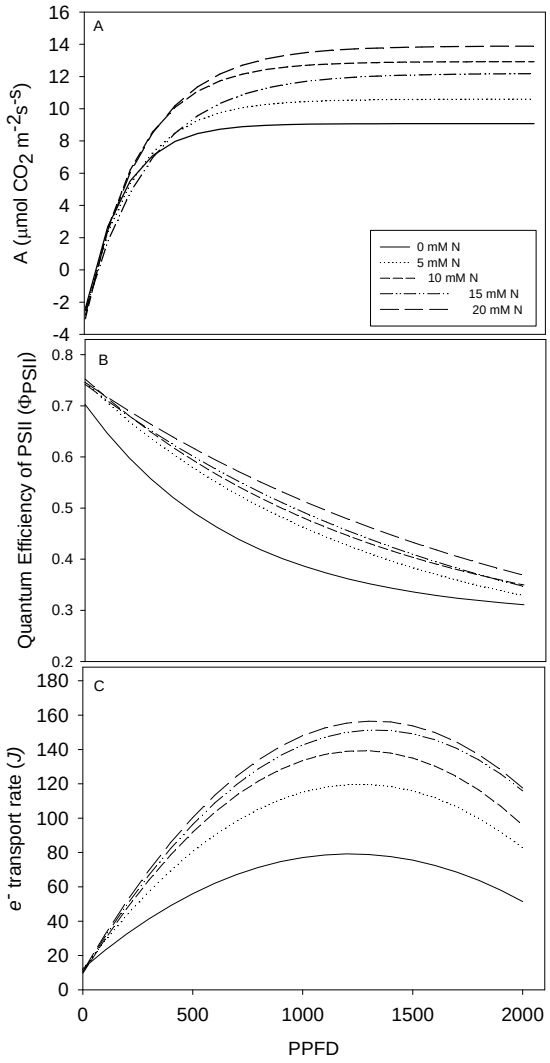

Figure 1-5. Light response curves of (A) Photosynthesis (A), (B) quantum efficiency of ΦPSII and (C) electron transport rate (J) of potted 'Jewel' strawberry plants grown under 5 different N fertilizer treatments (0, 5, 10, 15, 20 mM N).

Regression Equations: (A) 0N, $y=9.07-11.46^{(-0.0059*PAR)}$ ($R^2=0.83$, $P<0.0001$); 5N, $y=10.59-13.05^{(-0.0046*PAR)}$ ($R^2=0.90$, $P<0.001$); 10N, $y=12.92-15.95^{(-0.0043*PAR)}$ ($R^2=0.91$, $P<0.0001$); 15N, $y=12.19-14.72^{(-0.0035*PAR)}$ ($R^2=0.89$, $P<0.0001$); 20N, $y=13.89-16.69^{(-0.0038*PAR)}$ ($R^2=0.91$, $P<0.0001$). (B) 0N, $y=0.41^{(0.0014*PAR)}+0.29$ ($R^2=0.92$, $P<.0001$); 5N, $y=0.53^{(0.00076*PAR)}+0.21$ ($R^2=0.96$, $P<.0001$); 10N, $y=0.59^{(0.00053*PAR)}+0.14$ ($R^2=0.96$, $P<.0001$); 15N, $y=0.59^{(0.00056*PAR)}+0.15$ ($R^2=0.96$, $P<.0001$); 20N, $y=0.61^{(0.00048*PAR)}+0.13$ ($R^2=0.96$, $P<.0001$) (C) 0N, $y=38.76+0.041*PAR-0.000045*(PAR-764.10)^2$ ($R^2=0.84$, $P<0.0001$); 5N, $y=54.92+0.063*PAR-0.000068*(PAR-795)^2$ ($R^2=0.89$, $P<.0001$); 10N, $y=58.73+0.079*PAR-0.000081*(PAR-779.41)^2$ ($R^2=0.94$, $P<.0001$); 15N, $y=60.59+0.085*PAR-0.000079*(PAR-795)^2$, ($R^2=0.93$), $P<.0001$); 20N=$65.94+0.085*PAR-0.000084*(PAR-811.22)^2$ ($R^2=0.96$, $P<.0001$)

Light response curves showed an increase in maximum CO_2 assimilation (A_{CO2}) with increasing N content. However, the increases were primarily below 10mM N. N treatment had little impact on the initial slope of the curve (Figure 1-5A). Light adapted quantum efficiency of PSII (ΦPSII) response curves showed a greater response to N between the 0 mM N treatment and all other treatments even at the lowest light levels. N response among the other treatments was not observed until light levels were greater than 250 μmol $m^{-2}s^{-1}$ (Figure 1-5B)

A_{CO2} response curves to increasing internal CO_2 concentrations (Ci) showed trends similar to the light response curves with an increase in A_{CO2} with increasing N. However, increasing N application beyond 15mM N did not lead to further increases in A_{CO2} and both ΦPSII and electron transport (J) curves indicate a decline in the 20mM treatment. The initial slope of the A_{CO2}/Ci, ΦPSII and J curves was the steepest at the 15mM N applications (Figure 1-6). The ΦPSII curves indicate that the N content of the leaves affected the ability to utilize intercepted light at all levels of CO_2; however, the response was more pronounced at saturating CO_2 levels (Figure 1-6B).

Enzyme Activity. Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) both showed an increase in activity with increasing N treatment when expressed on an area basis (Figure 1-7A,B). However, when expressed on a N content basis, the activity of both enzymes increased from the 0N to 10N treatments but did not show further increases with additional N (Figure 1-7E,F). Fructose 1,6-bisphosphotase (FBPase) showed an increasing trend in activity when expressed on a leaf area basis (Figure 1-7B), and showed a significant decline in activity when expressed on a N content basis (Figure 1-7G). N treatment had no effect on sucrose phosphate synthase (SPS) activity when expressed on a leaf area or N content basis (Figure 1-7D, H).

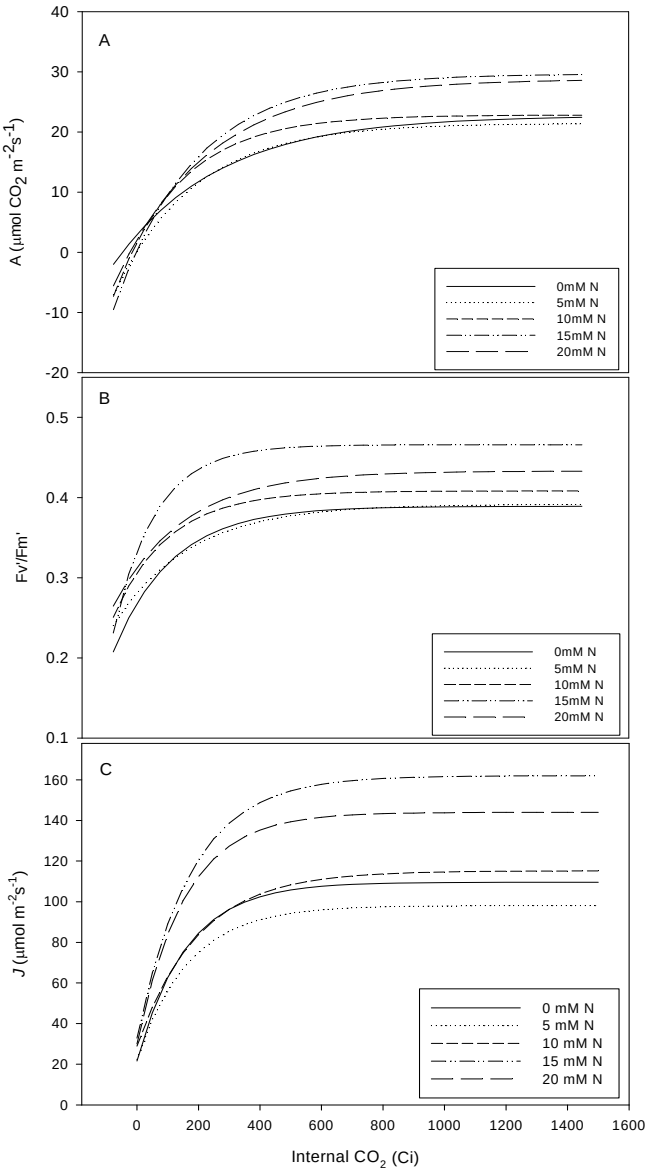

Figure 1-6 Internal CO_2 response curves of (A) photosynthesis, (B) quantum efficiency (ΦPSII) and (C) electron transport rate (*J*) of potted 'Jewel' plants grown under 5 different N treatments (0, 5, 10, 15, 20mM N).
Regression Equations: (A) 0N, $y=22.73-24.74^{(-0.0030*Ci)}$ ($R^2=0.90$, P<.0001); 5N, $y=22.45-28.48^{(-0.0039*Ci)}$ ($R^2=0.93$, P<.0001); 10N, $y=22.82-30.15^{(-0.0047*Ci)}$ ($R^2=0.98$, P<.0001); 15N, $y=29.66-39.20^{(-0.0039*Ci)}$ ($R^2=0.91$, P<.0001); 20N, $y=28.80-34.37^{(-0.0034*Ci)}$ ($R^2=0.96$, P<.0001) (B) 0N $y=0.39-0.18^{(-0.0053*Ci)}$ ($R^2=.70$, P<.0001); 5N, $y=0.39-0.15^{(-0.0041*Ci)}$ ($R^2=0.65$, P<.0001); 10N, $y=0.41-0.16^{(-0.0057*Ci)}$ ($R^2=0.68$, P<.0001); 15N, $y=0.467-0.23^{(-0.0075*Ci)}$ ($R^2=0.66$, P<.0001); 20N, $y=0.43-0.17^{(-0.00441*Ci)}$ ($R^2=0.82$, P<.0001). (C) 0N, $y=109.6-87.98^{(-0.00628*Ci)}$ ($R^2=0.81$, P<.0001); 5N, $y=98.11-75.66^{(-0.00597*Ci)}$ ($R^2=0.74$, P<.0001); 10N, $y=115.2-86.29^{(-0.00505*Ci)}$ ($R^2=0.96$, P<.0001); 15N, $y=162.0-129.10^{(-0.0057*Ci)}$ (R=0.55, P<.0001); 20N, $y=144.40-114.90^{(-0.00642*Ci)}$ ($R^2=.85$, P<.0001)

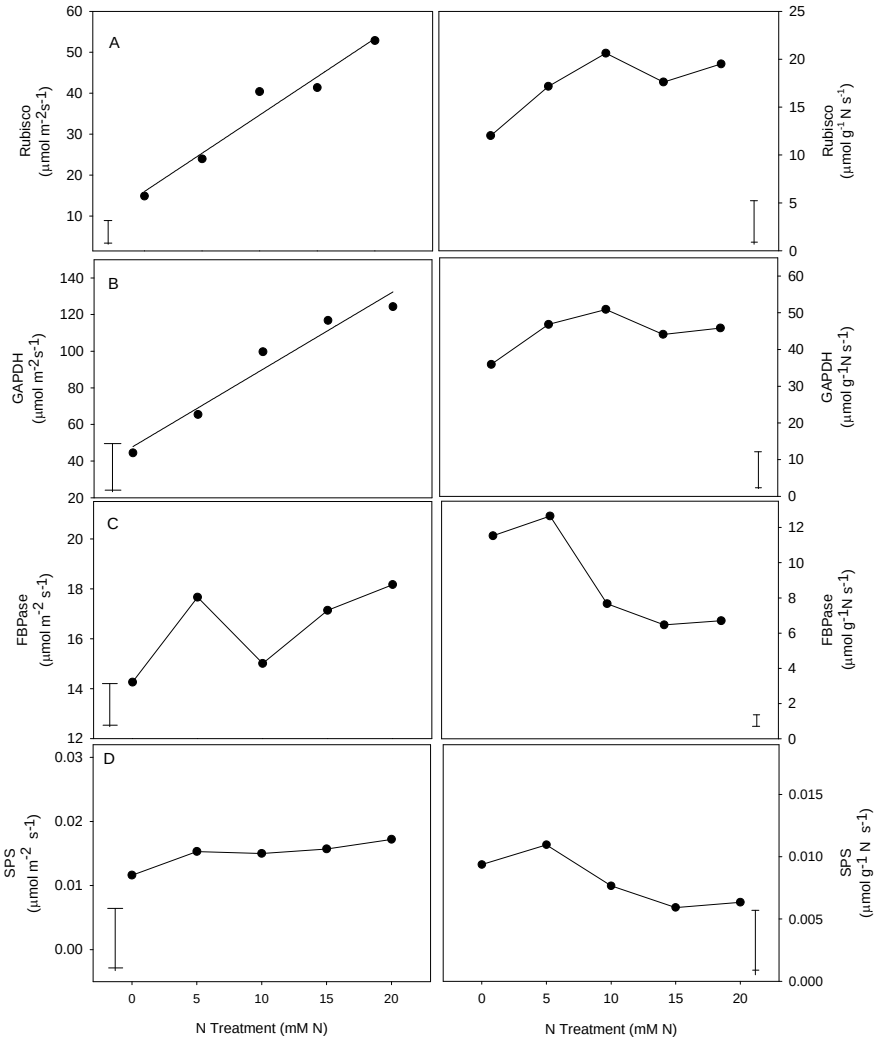

Figure 1-7. Effect of N on activity rates of enzymes involved in photosynthetic metabolism; Rubisco (A), GAPDH (B), and FBPase (C), SPS (D) on a leaf area basis (A-D) and on a leaf N content basis (E-H) of 'Jewel' plants grown in pots under different N treatments (0mM, 5mM, 10mM, 15mM and 20mM). Standard error indicated by error bar

Non-structural carbohydrates. N treatment did not affect the starch accumulation that occurred during the day; however, there was a significant increase in starch degradation (dusk-predawn content) with increasing N treatments (Figure 1-8). Glucose, fructose and sucrose content of the leaves were higher at dusk compared to predawn, indicating accumulation during the day and degradation of sugars during the night. Glucose and fructose content showed a slight increase or no change with increasing N content until the 20 mM treatment which resulted in a decrease in accumulation. The export of

sugars (dusk-predawn) showed a similar trend with a decreased amount of export at higher rates. Sucrose, the primary transport sugar, showed a sharp increase between the 0 and 5 mM treatment but then remained unchanged at higher rates of N. The export pattern of sucrose was different from fructose and glucose in that there was a slight increase in export at the higher N treatments (Figure 1-9).

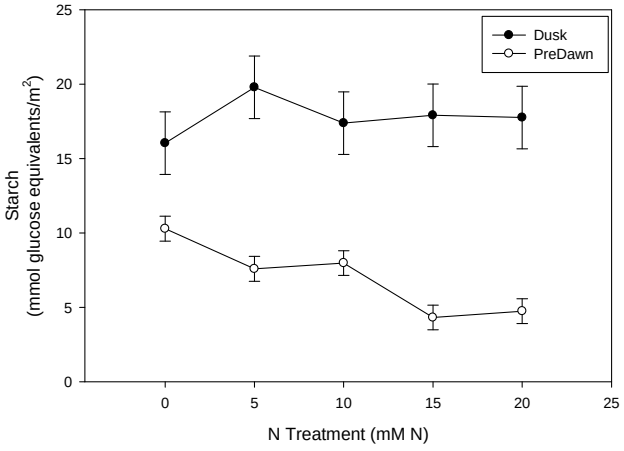

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Figure 1-8. Effect of nitrogen on leaf starch content samples taken at dusk and pre-dawn (after 9 hour dark period).

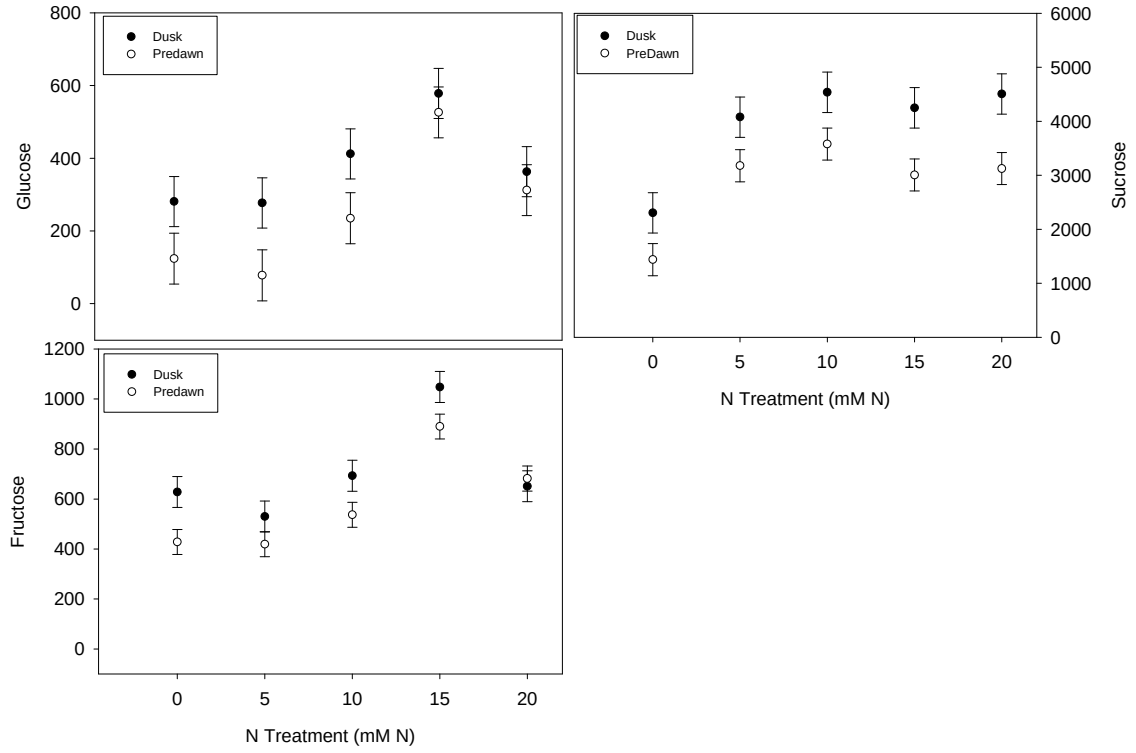

Figure 1-9. Concentration of sugars measured at dusk (open circles) and predawn (closed circles) of 'Jewel' leaves from plants grown under different N treatments**.**

Discussion

The relationship between N and photosynthesis has been extensively studied in several different species (Lawlor 2002; Reich et al. 1998a). N plays a role in A_{CO2} through direct interaction with the photosynthetic process as it is a major constituent of photosynthetic proteins such as Rubisco and a signaling molecule influencing enzyme activity (Champigny and Foyer 1992). It can also influence A_{CO2} through indirect effects on plant growth, source:sink ratio (Levin et al. 1989) and leaf morphology (Yoshida et al. 1969). The effect of N on A_{CO2} also is influenced by external conditions such as light intensity, CO_2 concentration, water supply, interactions of other nutrients, or physical factors such as lodging due to excessive growth (MacLeod 1969). If external factors are controlled, then data from growth analysis, chlorophyll

fluorescence, gas exchange, enzyme activity and end product utilization can be used to identify which biochemical factors are most important in regulating A_{CO2} under a given set of conditions.

The response of plant growth to N is well understood (Marschner 2002). Typically, there is an increase in shoot:root ratio due to increased shoot growth and decreased root growth with increasing rates of N application (Stitt 1999). In the current study, there was no significant effect of N on shoot:root ratio even though there was a slight decrease in root growth at the high N treatment (Figure 1-1). This observation may have been due to the timing of growth assessment which was done in the fall. During cool temperatures and short days of fall, there is increased partitioning of assimilates to the roots as well as translocation of resources from the leaves to roots (May and Pritts 1994). This reallocation may have masked the typical effect of decreased root growth at high N rates.

An increase in N content often leads to an increase in leaf size and a decrease in specific leaf weight (SLW) (Wright et al. 2001). The current study showed a curvilinear response of leaf size to increasing N until 15mM N (Figure 1-2). SLW did not show a response to N treatment (data not shown. This increase in leaf size may lead to increased light interception in the top layer of leaves; however, there is typically a dropping of leaf edges correlated with increasing leaf size which reduces the light interception (Marschner 2002). In addition, a dense canopy will cause increased shading in the interior, causing a greater proportion of the canopy to become a carbon sink. Although the shoot:root ratio did not change much, the increased number of interior leaves on the high N plants would increase the sink demand on the plant which could lead to higher A_{CO2}.

The effects of N supply on fruit yield are not completely understood; high N availability can increase yield due to increased source; however, this also must be

accompanied by adequate carbon supply and allocation to yield components. If carbon supply is not sufficient or allocation to harvested components is not maximized, increased yields will not be realized (Acuna-Maldonado and Pritts 2008; Voth et al. 1967). In the current study there was no effect of N on yield which may have been due to timing of N application; previous work has shown that spring applied N has minimal effect on yield in strawberries (Acuna-Maldonado and Pritts 2008). As fruit are one of the largest sinks on a strawberry plant, high fruit loads can lead to an increase in A_{CO2} (Forney and Breen 1985). However, it is not likely that yield influenced observed A_{CO2} in this study as gas exchange data were collected after the fruit had been removed.

There is a close relationship between leaf N content and photosynthesis as the majority of leaf N is found in proteins of the Calvin cycle and thylakoids related to CO_2 assimilation (Evans 1989). A curvilinear response of A_{CO2} to increasing N has been observed in several species including strawberry (Acuna-Maldonado and Pritts 2008), apple (Cheng and Fuchigami 2000) wheat (Evans 1983) and several other species (DeJong and Doyle 1985). This curvilinear response also was observed in several of the photosynthetic variables quantified in this study, with reduced effects of N beyond the sufficiency level (10-15mM).

Nitrogen limited plants had reduced maximum quantum efficiency of PSII (Fv/Fm) (Figure 1-4). Typically, Fv/Fm shows very little variation in un-stressed plants across taxonomic groups and as a result, this fluorescence variable has been used as a sensitive quantitative indicator of the effects of stress (Demmig and Björkman 1987). Fv/Fm values are decreased due to photoinhibitory damage to the PSII reaction center (Baker and Oxborough 2004). Under stress conditions, the protection mechanisms in place (i.e. xanthophyll and water-water cycle) are not sufficient to dissipate excess light energy leading to the formation of reactive oxygen species which cause damage to the D1 protein in PSII. It has been suggested that the thylakoid pH gradient required for

zeaxanthin heat dissipation may be maintained by reverse proton pumping by ATPase (Adams III et al. 1994). A reduction in realized quantum efficiency of PSII (Φ_{PSII}) has also been correlated with Fv/Fm (Björkman and Demmig 1987) which was also observed in this study (Figure 1-5).

Under moderate to high light conditions A_{CO2} /light response curves showed a significant response to N treatment (Figure 1-5A), with the low N treatments having a lower light saturation point and a lower maximum A_{CO2}. Response curves of Fv'/Fm' to increasing PPFD showed a reduction in Fv'/Fm' in response to light and an increase with N treatment, indicating that low N plants had a greatly reduced capacity to partition excitation energy to the light harvesting complex of PSII. (Figure 1-5B).

There was a significant reduction in the rate of electron transport (*J*) in response to N treatment, particularly under high light conditions (Figure 1-5C). At low light levels, one would expect that *J* would be limited by light and would therefore be unaffected by N treatment. At light levels below 250 μmol $m^{-2}s^{-1}$ the slope of the *J* response curve increased with increasing N until 10mM N; a response not reflected in the A_{CO2} curve (Figure 1-5A, C). Although there was a minor underestimation of the initial slope on the quadratic regression line, the observed data still indicate that there was an increase in *J* with increasing N content. Contrary to most woody plants (Marschner 2002), nitrate assimilation in strawberry leaves has been shown to be two-fold higher in comparison to roots (Darnell and Stutte 2001). If nitrate assimilation was significantly lower in the N-deficient treatments, there would be reduced competition between C and N assimilating processes leading to a reduced rate of *J* in the low N treatments. However, this remains unclear as the activity of nitrate reductase has been observed to be unchanged by increasing N content (Darnell and Stutte 2001)

Under saturating light conditions, the limitation to A_{CO2} is no longer incident PFD, but either Rubisco activity, RuBP regeneration or Triose Phosphate utilization (Farquhar and Sharkey 1982; Sage 1990), processes which are all dependent on N availability. The plateau in A_{CO2} at saturating light levels and higher were accompanied by a decline in *J*. When *J* is in excess relative to Rubsico activity or Pi regeneration, it is down-regulated to maintain a balance between production and utilization of photosynthetic products (Sage 1990), therefore, under high light conditions the limitation to photosynthesis may be due to reduced Calvin cycle enzyme activity or TP utilization (Figure 1-6C).

A_{CO2} response curves to internal CO_2 (Ci) were generated under saturating light conditions (1500 μmol PPFD $m^{-2}s^{-1}$) therefore, light and stomatal effects are controlled for (Farquhar et al. 1980) since the initial slope of the A_{CO2}/Ci curve represents the increase in carboxylation with one unit increase in internal CO_2 concentration (Farquhar and Sharkey 1982). According to the biochemical model by Farquhar et al. (Farquhar et al. 1980), the A_{CO2}/Ci curve can be divided into two regions, the first being the low CO_2 region where Rubisco is limiting. In this study, the initial slope of the A_{CO2}/Ci curves increased with increasing N treatment indicating an increasing carboxylation rate until the 10 mM N treatment (Figure 1-6A) . This is consistent with the curvilinear response observed in Rubisco activity to leaf N (Figure 1-7A). Similar observations of this curvilinear response of A_{CO2} and Rubisco activity were made on apple leaves grown under a range of N conditions (Chen and Cheng 2004). According to the model, the primary limitation to A_{CO2} under low CO_2 conditions is likely reduced Rubsico activity (Farquhar et al. 1980) a conclusion supported by the low Rubisco activity measured in the low N treatments (Figure 1-7).

The second region of the curve is the plateau where limitation is due either to the regeneration rate of RuBP or the utilization of TP which is linked to regeneration of

inorganic phosphate (Pi) needed for photophosphorylation (Farquhar et al. 1980). When the sugar phosphates produced through the photosynthetic process are utilized, the Pi is recycled back into the chloroplast for use in photophosphorylation, a process regulated by the concentration of Pi in the chloroplast (Cockburn et al. 1967; Harold 1980). In conditions where there is limited utilization of triose phosphates, fructose-2,6-bisphosphate accumulates and down-regulates the activity of fructose bisphosphotase (FBPase) the enzyme involved in cleaving Pi (Paul and Foyer 2001). As a result, carbohydrate metabolism is shifted to starch production which would accumulate in the chloroplast. In the current study there was an observed decrease in the activity of FBPase activity (Figure 1-7G) with increasing N, but no significant decrease in sucrose phosphate synthase (SPS) activity (Figure 1-7H) reflected by the minimal changes observed in sucrose content (Figure 1-9c). Although there was some indication of down-regulation of FBPase, it was not sufficient to affect sucrose production. This is similar to N responses observed in grape leaves (Chen & Cheng, 2003).

There was no increase in accumulated starch by the end of the light period with increasing N (Figure 1-8). Net production of starch was higher in the high N treatments, but it did not lead to accumulation due to increased export rates suggesting a sufficient sink demand for photosynthetic products. Previous work on strawberry has demonstrated a quadratic response of starch accumulation to increasing N (Acuna-Maldonado and Pritts 2008). As with the previous study, the low A_{CO2} observed in low N treatments was not due to starch accumulation which can suppress photosynthesis. Therefore, the starch levels likely did not have any influence on the low A_{CO2} observed in N-limited plants.

A_{CO2} can be decreased in the presence of high soluble sugars through repression of photosynthetic genes, particularly the expression of Rubisco (Iglesias et al. 2001;

Krapp et al. 1993a). Soluble sugar accumulation occurs when the plant is 'sink-limited'. Fruit are the strongest sink in a plant and can account for up to 40% of the dry matter (May et al. 1994); however, photosynthetic data were collected after fruiting so would not have been affected by fruit sink capacity. The roots are also a large sink, although there was no significant change in root:shoot ratio with N treatments. Further support that the low A_{CO2} in low N treatments was not due to feedback inhibition by soluble sugar accumulation is that the content of glucose, fructose and sucrose either decreased or did not change with decreasing N (Figure 1-9). This was also observed in previous studies where starch and total nonstructural carbohydrates were lower in the low N treatments (Acuna-Maldonado and Pritts 2008). Therefore, decreased rates of Rubisco and GAPDH observed in this study were likely not due to reduced gene expression as a result of soluble sugar accumulation. It is unlikely that feedback inhibition played a role at the higher N treatments as higher amounts of accumulation at dusk were accompanied by higher rates of degradation (Figure 1-9).

The photosynthetic capacity of strawberry plants reached a maximum when supplied with 10-15mM N with a significant reduction in plants grown with lower N supply. The reduced photosynthesis observed in the low N treatments was not due to feedback mechanisms as they were not accompanied by starch or soluble sugar accumulation. Therefore, we can conclude that the reduced photosynthetic capacity is a result of reduced activity of key photosynthetic enzymes due to limited N supply.

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CHAPTER 2

UNDERSTANDING THE BASIS OF DIFFERENCES IN PHOTOSYNTHETIC CHARACTERISTICS OF THE PROGENITOR STRAWBERRY SPECIES *F. VIRGINIANA* AND *F. CHILOENSIS.*

Introduction

The genus *Fragaria* consists of a complex series of polypoids (2x, 4x, 6x, 8x) that is found across the temperate regions of the globe. The cultivated strawberry, *Fragaria x ananassa* Duch. is a hybrid between two octoploid species; *F. chiloensis* Duch. and *F. virginiana* Duch. that was accidentally discovered in European gardens in the 18th century (Darrow 1966). This hybrid has been the basis of breeding programs since then and as a result, the genetic diversity of the cultivated strawberry is quite narrow (Dale and Sjulin 1990; Hancock and Luby 1993; Sjulin and Dale 1987). The majority of North American cultivars are derived from 53 founding clones, 7 of which account for 50% of the genetic makeup of the cultivar germplasm (Luby et al. 1991). This diversity is further reduced when cultivars for a particular production region are considered. In the northeastern U.S., only four founding clones account for half of the genetic contribution to the major cultivars (Luby et al. 1991).

Deleterious effects of inbreeding and genetic vulnerability to disease, pests and environmental stresses are potential problems with a narrow germplasm base (Luby et al. 1991). Breeding efforts in the northeastern U.S. have been focused on disease resistance to a greater extent than many other regions. However, compared to the rest of the U.S., yields in the Northeast have remained fairly constant over the past thirty years (Bertelson 1995) and the top performing cultivars have remained the same since

the late 1970s and mid 1980s (Hokanson and Finn 2000). Therefore, while there is a greater degree of disease resistance in the northeastern germplasm, the lack of diversity may be limiting improvements in productivity.

Limited genetic diversity has prompted researchers to investigate the potential of utilizing wild germplasm in modern breeding programs (Hancock 1991; Hancock et al. 1999; Hancock et al. 1989; Hancock et al. 2002; Marta et al. 2004; Sangiacomo and Sullivan 1992). Due to the difficulty of interspecific hybridization between the octoploid *F. x ananassa* and the lower ploidy *Fragaria* species, much of the work on wild species has been focused on the progenitor species which have been used to incorporate disease resistance (Jones 1966) and day neutrality (Hancock and Bringhurst 1978); however, wild germplasm use has been limited.

One aspect that has been evaluated is the potential to increase the photosynthetic capacity of the cultivated strawberry through breeding efforts with wild species. This is of particular interest in northeastern breeding programs where there have been minimal improvements in productivity of cultivars during the past three decades.

Studies evaluating the photosynthetic characteristics of *Fragaria* indicate that rates of some wild species are higher compared to those of the cultivated strawberry (Cameron and Hartley 1990; Chabot and Chabot 1977). When rates are expressed on a leaf area basis, *F. chiloensis* has been shown to have higher rates of photosynthesis compared to *F. virginiana* and the cultivated strawberry has rates that are intermediate to these two species (Hancock 1991; Hancock et al. 1989; Sedat et al. 1989). Rates of *F. chiloensis* have been reported to be 25-69% higher than those of the cultivar 'Totem' when expressed on a leaf area basis (Cameron and Hartley 1990). Studies on *F. chiloensis* x *F. x ananassa* hybrids have indicated that the photosynthetic characteristics of *F. chiloensis* is a quantitatively heritable trait (Hancock et al. 1989). While these

studies do suggest that *F. chiloensis* has higher photosynthetic potential and that this characteristic may be a heritable trait, the photosynthetic data have only been reported on a leaf area basis. The two progenitor species have very different leaf and plant morphologies and this may be affecting the reported rates of photosynthesis.

While suggestion has been made that utilization of *F. chiloensis* in breeding efforts could increase the photosynthetic capacity of the cultivated strawberry (Hancock 1991), no studies have gone beyond gas exchange measurements made on a leaf area basis. In order to effectively utilize this germplasm, or understand if greater carbon fixation can be genetically transmitted, it will be necessary to evaluate photosynthetic capacity while considering differences in morphology, N content and biochemical variables.

Response to varying N rates is a tool that can help separate components of photosynthesis. The objective of this work was to expose the strawberry progenitor species to varying levels of N, and then determine which components of photosynthesis express limitations.

Materials and Methods

Plant Material. Unrooted runners were provided by the National Clonal Germplasm Respository (Corvallis, OR) for two species: *Fragaria chiloensis* ssp. Yaquinta Bay (PI 551455) collected from Yaquinta Bay State Park in Oregon and *Fragaria virginiana*. LH-50 (PI 612495) which was collected from Lewis and Clark National Forest in Montana. Runners were rooted and propagated in the greenhouse to achieve adequate numbers. One year-old rooted runners were transplanted on 5 May 2007 to 4.4 liter pots filled with a 1:1 mix of sand:Metro Mix® (Scott's Co., Marysville,

Ohio). The plants were grown under natural conditions at the Cornell Orchards in Ithaca, New York, USA. (Lat. 42.44 N, Long.76.50 W)

Treatments. After planting, all plants were given one application of Hogland's solution with 10mM N, and then watered for 2 weeks before treatments began. Five different N treatments of a modified Hoagland Solution (Cheng and Fuchigami, 2000) with an N (NH_4NO_3) concentration of 0, 5, 10, 15 or 20 mM were applied twice a week for the duration of the experiment. The experiment was designed as a randomized complete block experiment with 5 blocks and 5 replications per treatment in each block.

Gas Exchange Data. Gas exchange data were collected using the Li-6400 (Li-Cor Inc., Lincoln, Neb.) infrared gas analyzer. Conditions were constant for all measurements; ambient CO_2 (360μmol mol^{-1}), photosynthetic photon flux density (PPFD) of 1300 μmol $m^{-2}s^{-1}$, temperature and water vapor pressure were maintained at $25^oC \pm 1^oC$ and 2.3 kPa, respectively. Light adapted measurements of CO_2 assimilation (A_{CO2}) were made once a week on a recently expanded leaf between 10:00-14:00. A_{CO2}-Ci curves with CO_2 concentrations ranging from 45ppm to 1500 ppm were generated on a recently expanded leaf of three plants per treatment and data for each treatment were pooled. Dark respiration measurements were made on a recently expanded leaf during predawn hours after a seven hour dark period.

Chlorophyll Fluorescence. Chlorophyll fluorescence was measured using the Li-6400 infrared gas analyzer with the chlorophyll fluorescence head attached to allow simultaneous gas exchange and fluorescence measurements. Measurements were made under full sunlight at noon to attain data of the fluorescence yield of leaves adapted to ambient light conditions (F) and the maximum light-adapted fluorescence yield when exposed to a supersaturating pulse of light (F_m'). These parameters were

then used to calculate the effective quantum yield of PSII ($Fv'/Fm'=F_m'-F/F_m'$). Predawn measurements were made to determine the dark-adapted minimal fluorescence (F_o).

Calvin Cycle Enzymes. Ten $1cm^2$ leaf disks were collected from three plants in each experimental unit at noon under full light (PFD 1700 PAR μmol $m^{-2}s^{-1}$) and immediately placed in liquid N_2. Material was stored at -80°C until the time of the assay. Rubisco, NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC1.2.1.12) and fructose-1,6-bisphosphotase (FBPase, EC3.1.3.11)were extracted according to protocol described by (Chen and Cheng 2003). Rubisco, GAPDH and FBPase extractions were carried out using a total of three leaf disks. In a precooled mortar and pestle, the leaves were ground in 1.5mL of a buffer solution containing 50 mM Hepes-KOH, 10mM $MgCl_2$, 2mM ethylenediaminetetraacetic acid (EDTA), 10mM DTT, 1%(v/v) Triton X-100, 5% (w/v) bovine serum albumin (BSA), 10% (v/v) glycerol, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged for 5 mins at 13,000rpm and the supernatant was used immediately for spectrophotometric analysis of enzyme activity.

Non-structural Carbohydrates. Starch, glucose, sucrose and fructose were measured from leaf discs that were sampled at dusk, after a 15-hour photoperiod and again from the same leaf at pre-dawn after approximately 8 hours of dark. Leaf discs were punched with a one cm^2 punch and immediately placed in liquid N_2 and stored at -80°C until analysis. Starch measurement was carried out according to Chen and Cheng (2003), extraction was carried out by grinding three frozen leaf discs in four ml 80% ethanol, placing homogenate in a 80°C water bath for 30 minutes and then centrifuging at 13,000 rpm for five minutes. Tissue was re-extracted two more times and supernatants were combined. After supernatant was removed, the remaining pellet was boiled in two mL of 0.2 M KOH for 30 mins then the pH was adjusted to 4.5 with 1M

acetic acid. Digestion of starch was then carried out by adding 50 units of amyloglucosidase (EC3.2.1.3) at 55°C for 30 min, then stopping the reaction by placing in boiling water for one minute, cooling and diluting to 5 mL with H_2O_2 and centrifuging for 5 mins. Supernatant was then used for starch spectrophotometric measurements at 340nm (Jones, 1977) to analyze products of starch hydrolysis.

Leaf Nitrogen and Carbon. Duplicate samples from each experimental unit of approximately 200mg dried leaf tissue were used to analyze the N content of the leaves. Analysis was carried out using the LECO C/N analyzer (Leco Inc., St. Joseph's, MI).

Growth Analysis. At the end of the trial, plants from the 0, 10 and 20 mM N treatments were destructively harvested, divided into components, dried at 70°C to obtain dry weights.

Results

Overall, *F. virginiana* was larger than *F. chiloensis* (Fig.2-1). Specific leaf weight (SLW) of both species showed no response to N levels; *F. chiloensis* had higher overall SLW (Table 2-1). The root:shoot ratio in both species decreased with increasing N; however, the response was much greater in *F. chiloensis* due to the increased shoot

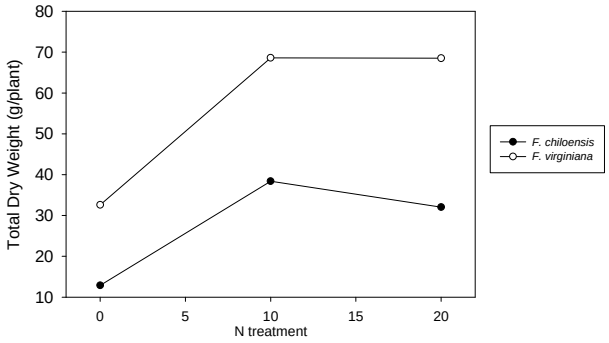

Figure 2-1 Total dry weight of *F. chiloensis* and *F. virginiana* grown under three different N treatments.

growth with increasing N. In contrast, *F. virginiana* had a much greater response in root growth to increasing N so the change in root:shoot ratio was smaller in this species. Both species had a similar response of total plant dry weight (PDW) to increasing N with the greatest response seen between the 0 and 10 mM N treatments and no further response with additional N (Figure 2-1).

Table 2-1 Root, crown, shoot, root:shoot ratio, specific leaf weight (SLW) of *Fragaria* species grown under 3 different N treatments (0, 10, 20 mM N).

N Treatment	Root DW (g/plant)	Crown DW (g/plant)	Shoot DW* (g/plant)	Root:Shoot	SLW (kg/m^2)
F. virginiana					
0	5.7	3.1	21.5	0.27	0.060
10	11.6	5.2	41.4	0.26	0.076
20	11.9	5.1	47.3	0.25	0.083
Linear Regression (p-value)	*0.022*	*0.0086*	*<.0001*	*NS*	*NS*
R^2	0.32	0.40	0.83		
Quadratic Regression (p-value)	*0.0335*	*0.0044*	*<.0001*	*NS*	*NS*
R^2	0.41	0.57	0.90		
F. chiloensis					
0	3.0	2.8	5.6	0.61	0.136
10	4.4	3.8	28.6	0.15	0.109
20	4.2	3.0	41.3	0.10	0.093
Linear Regression (p-value)	*NS*	*NS*	*<.0001*	*0.0012*	*NS*
R^2			0.94	0.60	
Quadratic Regression (p-value)	*NS*	*NS*	*<.0001*	*0.0008*	*NS*
R^2			0.97	0.73	

*shoot DW=leaf DW+runner DW

Both species showed a typical curvilinear response of leaf N content to increasing N treatments with *F. virginiana* having higher amounts of leaf N compared to *F. chiloensis* (Figure 2-2A). C/N ratio showed a curvilinear response to increasing N with an overall lower ratio in *F. virginiana* (Figure 2-2B)

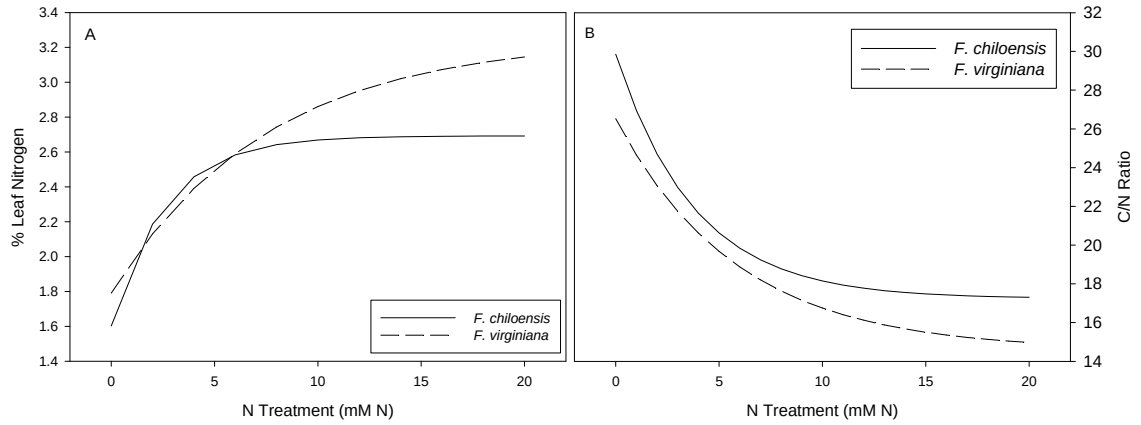

Figure 2-2. L eaf N content (A) and C/N ratio (B) of *F. chiloensis* and *F. virginiana* plants grown under different N treatments.
Regression equations: *F.chil* (%N) = =2.69-0.40$^{(-0.38*trt)}$, (R^2=0., P<.0001); *F.virg* (%N) = =3.25-0.45$^{(-0.13*trt)}$ (R^2=0., P<.0001). *F.chil* (C/N) =12.62$^{(-0.26*Trt)}$+17.24 (R^2=0.96, P<.0001); *F.virg* (C/N) =11.95$^{(-0.17*Trt)}$ +14.58 (R^2=0.84, P<.0001)

Gas Exchange and Chlorophyll Fluorescence. The species had different responses to the N treatments in the predawn measurements. *F. chiloensis* Fv/Fm values showed no response to increasing N; however, respiration rates increased almost two-fold with increasing N. *F. virginiana* showed the opposite response with no change in respiration rate but a significant increase in Fv/Fm with increasing N treatment (Figure 2-3). On an area basis, *F. chiloensis* had higher rates of A_{CO2}, *J* and Fv'/Fm' (Figure 2-4 A-C). *F. chiloensis* had steeper initial slopes on the A/Ci curves and showed a greater response in maximum rates to the N treatments compared to *F. virginiana* (Figure 2-4A). Similar trends were observed in electron transport rate (*J*). When evaluated on a leaf dry weight basis, the 0N treatment of *F. chiloensis* had the shallowest initial slope, and the lowest maximum A_{CO2} and the lowest *J* rate (Figure 2-4E). Expressing on a leaf area or mass basis did not change the *F. virginiana* results much. However, on both a leaf area and dry weight basis, the 10 and 20 mM N *F. chiloensis* had the highest overall rates (Figure 2-4D). N treatment did not have an effect on the Fv'/Fm' of *F.*

chiloensis; however, *F. virginiana* had lower rates of Fv'/Fm' in the 0 and 10 mM N treatments (Figure 3-4C).

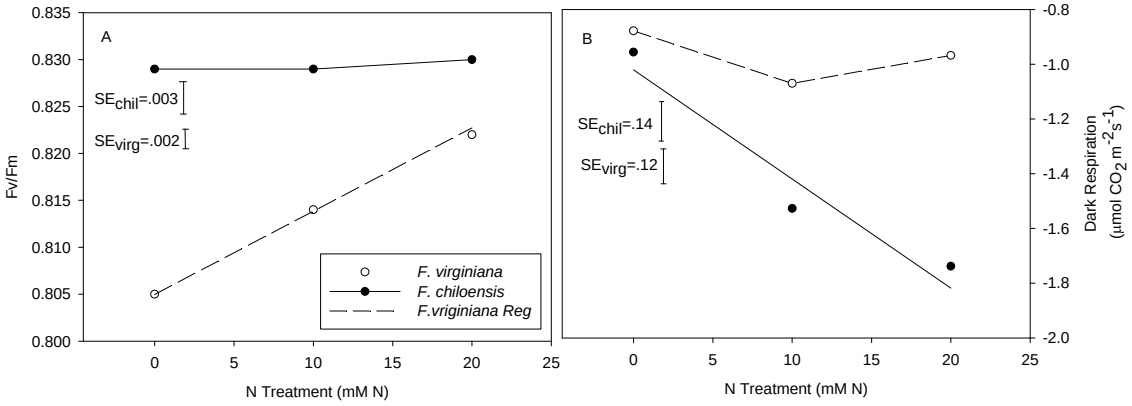

Figure 2-3 Predawn Fv/Fm (A) and dark respiration rates (B) of F. chiloensis (closed circles) and F. virginiana (open circles) under increasing N treatments (0,10,20 mM N)

Photosynthetic Enzymes *F. chiloensis* had higher activity on both area and weight basis except for the 0N *F. chiloensis* which had the lowest activity of Rubisco, GAPDH and PRK. Both species had similar increasing trends in Rubsico and GAPDH with higher N content and no significant response to N in PRK. FBPase (Figure 2-5) was much lower in the 0N treatment of *F. chiloensis* but did not increase with additional N after 5mM. *F. virginiana* showed no response to increasing N and had lower overall rates at all N levels compared to *F. chiloensis*.

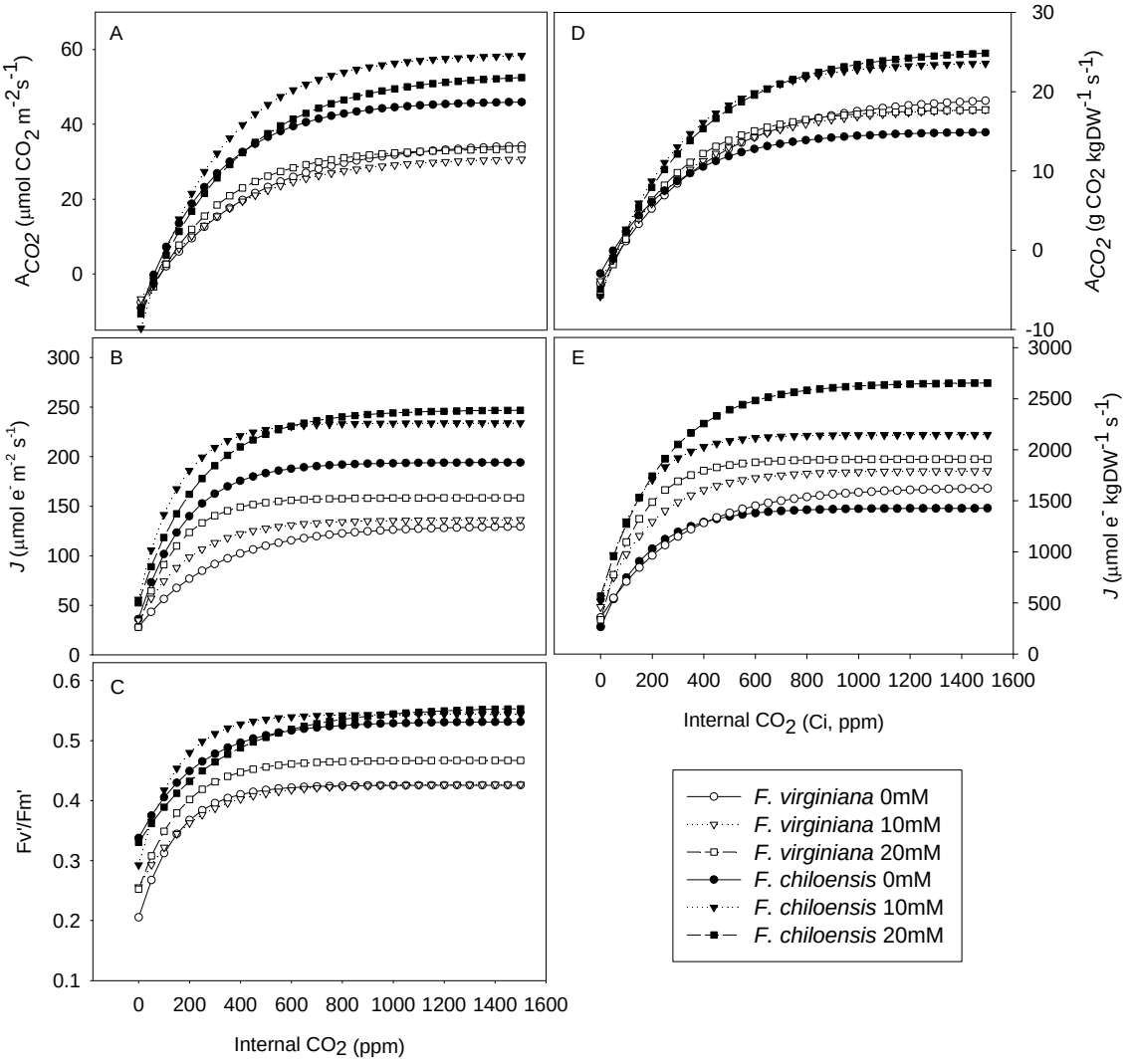

Figure 2-4. CO_2 response curves of CO_2assimilation (A, D), electron transport rate (*J*) (B, E) and (C) Fv'/Fm' of *F. chiloensis* and *F. virginiana* on a m^{-2} and kg dry weight (DW) basis, grown under different N treatments (0, 10 and 20 mM N)

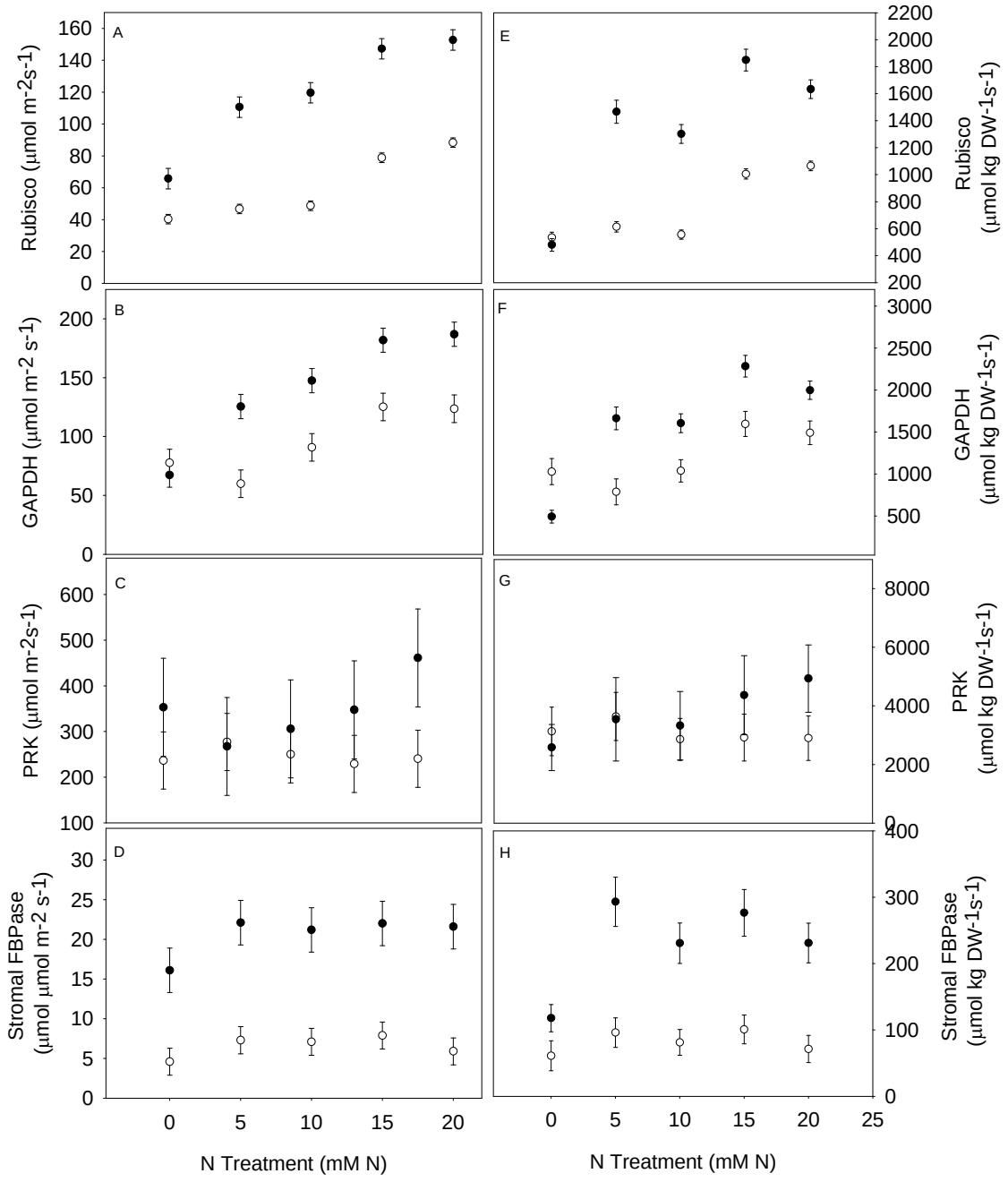

Figure 2-5 Activity of photosynthetic enzymes of *F. chiloensis* (closed circles) and *F. virginiana* (open circles) l

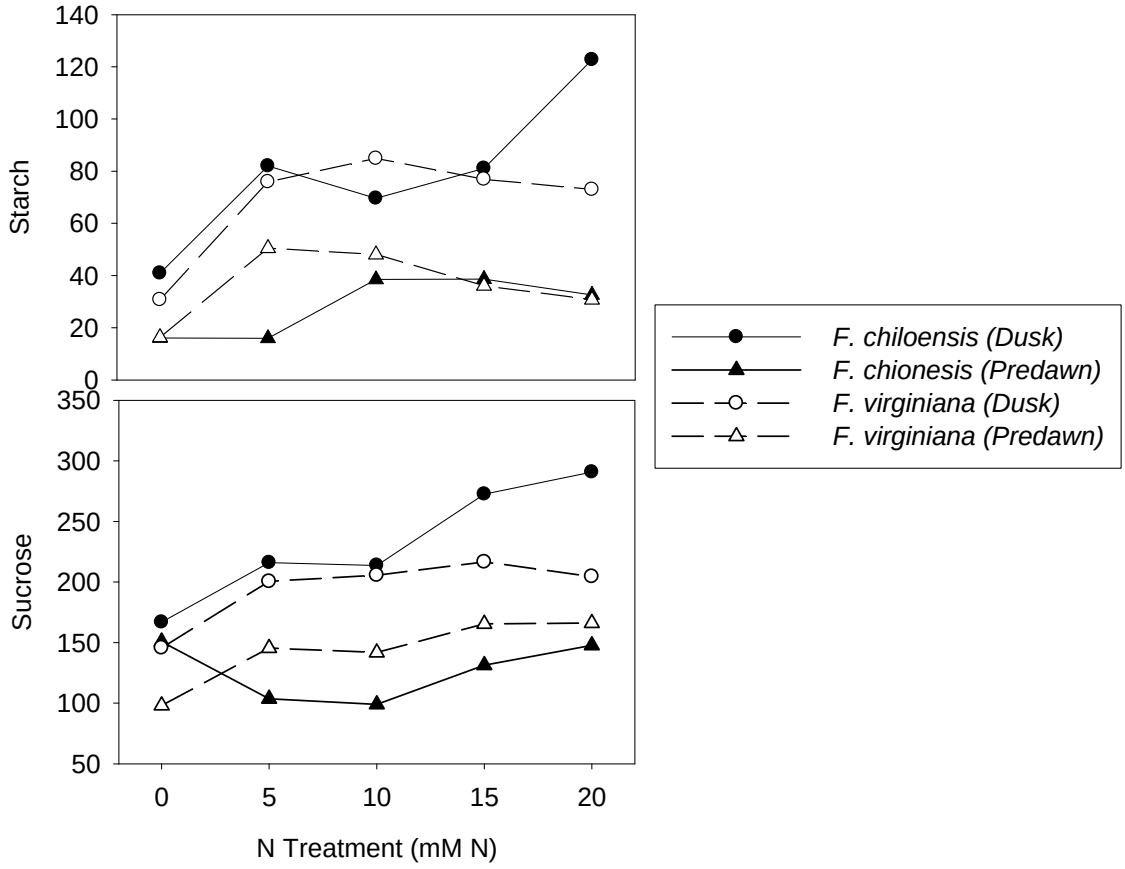

Figure 2-6 Dusk and predawn starch and sugar accumulations in leaves of *F. chiloensis* and *F. virginiana* grown under different N treatments**.**

Discussion

The progenitor species of the modern strawberry (*Fragaria x ananassa)*; *F. chiloensis* and *F. virginiana* have different ecological backgrounds. *F. chiloensis* is found in coastal regions of North America from California to Alaska and can be found in a range of habitats, from high light conditions in poor, sandy soils to woodland meadows that are higher in nutrients (Hancock and Bringhurst 1979; Moon et al. 1990). Clones found on sand dunes have been shown to have higher N accumulation under low N conditions and initiate more leaves and crowns and have a lower N compensation

point for photosynthesis compared to ecotypes from more fertile habitats (Moon et al. 1990). The genotype used in this study, *F. chiloensis* Yaquinta Bay, is from a vegetated area adjacent to the beach on the coast in Oregon. In contrast, *F. virginiana* is typically found in woodlands under more nutrient rich conditions (Darrow 1966). *F. virginiana* LH 50-4 was collected from a forest habitat in Montana. These different ecological backgrounds have significant effects on plant morphology and physiology which can have large effects on photosynthetic capacity. Leaf structure, N content, enzyme activity and source sink ratios are variables that must be considered when comparing the photosynthetic potential of the two species.

In the current study, *F. chiloensis* demonstrated higher photosynthetic capacity on an area basis (Figure 2-4). This is consistent with previous studies which have evaluated photosynthetic capacity of the progenitor species and found that on a leaf area basis, *F. chiloensis* has consistently higher rates compared to both *F. virginiana* and the cultivated strawberry which has rates intermediate to the two species (Cameron and Hartley 1990; Hancock et al. 1989; Hancock et al. 2002; Sedat et al. 1989). Due to the differences in plant morphology and ecological background of these species, the differences in gas exchange rates must be evaluated with consideration for variables such as: N content, leaf and plant morphology and biochemical processes, all of which can have large effects on photosynthetic capacity.

N content can affect photosynthesis directly as it is a constituent in photosynthetic proteins and indirectly through its effect on plant growth. N content has been highly correlated with A_{CO2} since Rubsico, the primary carboxylation enzyme, comprises the majority of soluble leaf protein (Chapin III et al. 1987). This is supported by studies that have shown that as leaf N increases, the proportion of N used for structural components remains the same, whereas the amount used in Calvin Cycle enzymes increases (Evans 1989). Plants were evaluated under a range of N conditions

in order to account for the effect of differential N content. Leaf N content increased in a typical curvilinear response in both species (Marschner 2002). *F. chiloensis* had a lower leaf N content at the high N treatments which was likely due to a dilution effect as a result of an almost six-fold decrease in root:shoot (R:S) ratio, primarily because of increased shoot growth (Table 2-1). A decrease in root:shoot ratio is a common response to increasing N availability (Marschner 2002). *F. virginiana* did not show any significant change in the R:S ratio as both root and shoot dry weight increased with N treatments.

Both species showed a similar trend in C/N ratio with a sharp decline between 0 and 10mM N and then leveling off, a response mirroring the leaf N content. The relationship between C and N can vary depending on photosynthetic activity of the plant. If there is sufficient Rubisco available to the plant, an increase in N will lead to a reduction in the amount of C because of the C requirement of N assimilation. If Rubisco is limiting, an increase in N can lead to an increase in C status due to increased photosynthesis (Cheng and Fuchigami 2002). There was no significant change in %C in the leaves across the treatments; however, both species showed a slight increase in % C between 0 and 10mM N treatments and then a slight decline at the highest N treatment. Indicating that under the low N treatments, Rubisco may have been limiting. This is supported by the gas exchange data (Figure 2-4) and the enzyme data (Figure 2-5) of the 0N *F. chiloensis* plants. These had the lowest A_{CO2} rates on mass basis and also had the lowest Rubisco and GAPDH content.

Despite the lower % leaf N content, *F. chiloensis* (Figure 2-2A) had higher photosynthetic capacity (i.e. Fv/Fm, Fv'/Fm', A_{CO2} and J) than *F. virginiana* at all N levels except the 0N treatment (Figure 2-5). One possible explanation for this is that *F. chiloensis* is allocating a greater percentage of leaf N to soluble proteins (i.e. Rubisco, other Calvin cycle enzymes) (Evans 1989), an allocation pattern that has been shown to

differ in wheat (Evans 1983), rice (Makino et al. 1984) and spinach (Terashima and Evans 1988).

Previous studies have evaluated photosynthesis of *F. chiloensis* and *F. virginiana* on a leaf area basis (Cameron and Hartley 1990; Hancock et al. 1989). This can be misleading due to the large differences in SLW. Expressing photosynthetic rates on a leaf mass basis takes into account the variation in SLW (Poorter et al. 1990; Walters et al. 1993). When A_{CO2} and electron transport (*J*) were expressed on an area basis, *F. chiloensis* had consistently higher rates than *F. virginiana*; 68-94% higher under ambient CO_2 and 47-64% higher in saturating CO_2 (Figure 2-4 A,B). When expressed on a mass basis, the differences were not as pronounced, although *F. chiloensis* still had higher photosynthetic capacity under non-limiting N. Under ambient CO_2 conditions, A_{CO2} of *F. chiloensis* were 7% (0N), 47% (10N) and 24% (20N) higher compared to *F. virginiana.* Under saturating CO_2 conditions, differences between *F. chiloensis* and *F. virginiana* were -12% (0N), 34% (10N) and 33% (20N) (Figure 2-4 D,E). Although the differences in photosynthetic capacity may not be as large as previously reported, *F. chiloensis* does appear to have consistently higher rates compared to *F. virginiana.*

The two species showed very different responses to N in the dark adapted measurements of maximum quantum efficiency of PSII (Fv/Fm) and dark respiration. Fv/Fm values were significantly higher for *F. chiloensis* and were not affected by N status, compared to *F. virginiana* which had overall lower values that were strongly correlated with N content. A correlation between Fv/Fm and the light adapted maximum quantum efficiency of PSII (ΦPSII) exists (Björkman and Demmig 1987) and this was reflected in the current study. No response of ΦPSII to N was observed in *F. chiloensis* but an increase in the high N treatment of *F. virginiana* (Figure 2-4C) was found. Under N limiting conditions, synthesis of pigments associated with LHCII is

reduced resulting in reduced capacity to harvest light energy and partition it to photochemistry (Plumley and Schmidt 1989). These findings indicate that the basis for the higher rates of A_{CO2} in *F. chiloensis* can first be observed in the ability to more efficiently capture and partition absorbed excitation energy to photochemistry which is advantageous under low light conditions as would be observed inside the canopy.

In addition to a higher capacity to capture and partition light energy, *F. chiloensis* also appears to have an increased capacity to assimilate C. A/Ci curves of all treatments of *F. virginiana* had a shallower initial slope compared to the 10 and 20mM N *F. chiloensis*; however, the 0N treatment of *F. chiloensis* had the shallowest slope (Figure 2-4D) and lowest Rubisco activity (Figure 2-5E) over all. The initial slope of the A/Ci curve may be an indication of a Rubisco limitation (Farquhar et al. 1980). *F. virginiana* had a minimal change in slope from the 0 to 20 N treatments (Figure 3-4D), despite an increase in Rubisco activity in the 20mM N treatment (Figure 3-5E). This discrepancy between the A_{CO2} data and the enzyme data would suggest that the limitation to the photosynthesis is not a 'single factor' limitation as the model suggests. Several studies have suggested that the control of photosynthesis is co-regulated by additional factors such as diffusion of CO_2 through the leaf aqueous phase (Stitt 1991; Woodrow et al. 1990). Given the large difference in leaf morphology between the two species, this may be contributing to limitations. It has also been shown that control coefficients (contribution to photosynthetic limitation) of Rubisco in plants grown under high N conditions are much lower compared to plants grown under low N conditions (Stitt and Schultze 1994). This would explain why in both species, there was minimal change in A_{CO2} rates with increased N application beyond 10mM N.

Further information about photosynthetic limitations can be derived by evaluating electron transport rates (J), end product accumulation and enzyme activity. Under saturating CO_2 conditions, A_{CO2} can be limited by either low *J* or by insufficient

Pi regeneration linked to starch and sugar accumulation (Farquhar and Sharkey 1982; Stitt et al. 1990). The low A_{CO2} observed in the 0N *F. chiloensis* treatment was accompanied by significantly reduced activity of both GAPDH and FBPase as well as reduced rates of electron transport (Figure 2-4). The decrease in enzyme activity could be due either to low levels of the protein in the cell resulting from N limitations or due to down regulation as a result of sucrose accumulation (Iglesias et al. 2001). Starch accumulation was quite low during the day and most of the starch was degraded during the dark period. Although sucrose accumulation was low in the 0N treatment, there was minimal export of sucrose during the dark period. Under low N conditions, total dry matter accumulation in *F. chiloensis* is very low and may have resulted in an increased source:sink ratio and accumulation of soluble sugars which has been shown to repress photosynthetic genes (Krapp et al. 1993b). This is supported by the significantly lower rates of FBPase activity observed in the 0N treatment of *F. chiloensis* (Figure 3-5H) which is suppressed by sucrose accumulation. The 0N *F. chiloensis* treatment had the lowest *J* which could be a result of decreased thylakoid proteins due to N limitation (Evans 1983). However, considering the low export rates of sucrose and low activity of FBPase, it is likely that the activity of the thylakoid proteins has been down-regulated by the reduced triose phosphate utilization and subsequently reduced activity of Calvin Cycle enzymes (Sage 1990).

Increasing N beyond 10mM N did not lead to increased A_{CO2} rates which has been observed in previous studies on the cultivated strawberry (Acuna-Maldonado and Pritts 2008). Although there was no response of $A_{CO2,}$ there was a significant response of *J* to increasing N (Figure 2-4). *J* can be influenced by competing processes such as N assimilation, photorespiration, triose phosphate utilization or the Mehler reaction (Maxwell and Johnson 2000). This increase in the high N treatment could be due to the increased demand of N assimilation which is a significant alternative sink for electron

capacity under conditions of excess N availability (Kamis et al. 1990). If Rubsico were limiting in this situation, the increased demand of N assimilation would lead to a reduction in A_{Co2}, however, this was not observed. A similar, though less dramatic response was observed in *F. virginiana.*

There are several possible reasons for the lower photosynthetic capacity observed in *F. virginiana.* Despite having higher N content in the leaves, the activity of the Calvin Cycle enzymes are consistently lower compared to *F. chiloensis* (Figure 3-5E-H). However, the rate of enzyme activity is regulated by several processes; therefore, other variables such as, light harvesting capacity of LHCI which would have an effect under light-limiting conditions, partitioning of light energy to photochemistry and utilization of photosynthetic end products (Iglesias et al. 2001; Krapp et al. 1993b) must be considered. Under all N conditions, *F. virginiana* had lower Fv/Fm and Fv'/Fm' which can limit A_{CO2} through reduced light harvesting capacity and partitioning to photochemistry (Plumley and Schmidt 1989). This species is native to a lower light, woodland habitat so it could have evolved in a lower light harvesting capacity. However, under the highest N treatment, there was an increase in ΦPSII but no response in A_{CO2}, suggesting that there is another variable limiting $A_{CO2.}$

The activity of FBPase in *F. virginiana* was low and remained low at all N levels which can indicate that low rates of sucrose are being produced in the leaves of *F. virginiana* due to limitations in the Calvin Cycle, resulting in a reduced need for FBPase or feedback inhibition by sucrose accumulation, suggesting high source:sink ratios. Increasing N content led to increased shoot and root growth and subsequently, there was little change in the root:shoot ratio. Although there was a slight increase in the amount of starch degradation with increasing N, the change was minimal. There was also little change in the amount of sucrose accumulation and export with increasing N content. The constant rate of sucrose accumulation and export and the constant

starch accumulation and degradation could suggest that there is a limited supply of photosynthate. Alternatively, it can also indicate that the rate of photosynthesis is limited by the sink strength. Evaluation of the enzyme activity data shows that there is an increase in activity of Rubisco and GAPDH with increasing N; therefore, if the limitation was the activity of Calvin Cycle enzymes there would be a subsequent increase in A_{CO2}. Given the large canopy size of this species, the lower rates may be a result of being sink-limited.

This study has shown that the apparent rate of A_{CO2} of *F. chiloensis* does appear to be higher compared to *F. virginiana*. However, despite the high rates of carbon assimilation, *F. chiloensis* had significantly lower amounts of total dry weight. *F. virginiana* had total dry weights that were 65%, 37% and 25% greater than *F. chiloensis* under the 0, 10 and 20mM N treatments, respectively (Figure 2-1). The correlation between the rate of A_{C02} and plant carbon gain is influenced by A_{CO2} as well as consumption of C by competing processes such as respiration and loss of plant matter by physical damage or herbivory (Pearcy et al. 1987). This apparent lack of correlation between A_{CO2} and total plant carbon gain may be due to the high respiration rates measure on the 10 and 20 mM N treatments of *F. chiloensis* which were 52% and 74% higher than *F. virginiana* (Figure 2-3B). When considering utilizing this germplasm for increased productivity of the cultivated strawberry, the high rates of carbon assimilation must be considered along with increased carbon gain. The plants must also have desirable growth patterns and allocation of dry matter. Increases in carbon allocation will only benefit plant productivity if resources are allocated to yield components (i.e. crown, inflorescence and fruit). Under the 0 and 10mM N conditions, *F. chiloensis* showed greater partitioning of dry matter to the crowns (24.6% and 10.3%, respectively) compared to *F. virginiana* (10.2% and 8.9%, respectively). Crown dry weight has been correlated with yield in previous studies on strawberries (Strik and

Proctor 1988b) and this may prove to be a viable avenue for improvement. Further evaluation of photosynthetic capacity of *F. chiloensis* and hybrids with the cultivated strawberry are needed to determine the potential for increasing productivity of the cultivated strawberry through increased photosynthetic capacity.

In summary, the results of this study confirm previous findings that under non-limiting N conditions, *F. chiloensis* has significantly higher photosynthesis compared to *F. virginiana* which may be a result of increased activity of Calvin Cycle enzymes. However, the differences were not as great as previously reported when evaluated on a mass basis due to the difference in leaf morphology. The high dark respiration rates of *F. chiloensis* and the lack of correlation between dry matter accumulation and carbon assimilation rates may limit the use of this germplasm as a source for increased carbon assimilation. While previous studies have shown that the higher photosynthetic capacity can be inherited (Hancock et al. 1989), analysis of hybrids with the cultivated strawberry must be evaluated for total dry matter accumulation and respiration rate in order to determine the impact on plant carbon accumulation. Another aspect that may make the use of *F. chiloensis* desirable for use in breeding to improve germplasm is the indication that *F. chiloensis* may have higher amounts of dry matter partitioning to the crown and less to roots which is a key yield component. Due to the complexity of regulation processes of photosynthesis, it is difficult to determine the potential of this germplasm to increase photosynthetic capacity of the cultivated strawberry; however, this study does confirm previous findings and suggests that further investigation on hybrids with the cultivated strawberry could lead to a greater understanding of the potential.

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CHAPTER 3

WITHIN SPECIES VARIABILITY OF GROWTH AND PHOTOSYNTHETIC CHARACTERISTICS OF *F. CHILOENSIS* AND *F. VIRGINIANA*

Introduction

The cultivated strawberry (*F. x ananassa*) is a hybrid of two octoploid species: *F. virginiana* and *F. chiloensis* (Darrow 1966). *F. chiloensis* contains a wide variety of morphological and physiological diversity, with over 4000 genotypes among the collected accessions from ecologically diverse sites ranging from British Columbia down the west coast of the United States and in Chile (Dale and Luby 1991). Collection sites vary ecologically from sandy beaches at sea level, to inland meadows with heavy-textured soils, to volcanic mountain sides at high elevation. Although different accessions vary morphologically and physiologically; in general, this species is distinguished by its thick, leathery leaves with broadly rounded teeth and relatively stout runners. *F. chiloensis* has been identified for its superior disease resistance, pest resistance, stress tolerance, water use efficiency, low chilling, and high photosynthesis rates (Hancock 1999).

F. virginiana contains several genotypes among the collected accessions from sites mainly in the northeastern U.S., but populations are found in Oregon and Virginia as well as Ontario, Canada. In general this species is distinguished by having numerous slender trailing runners, slightly pubescent stems, and thin leaves that are strongly toothed (Darrow 1966). *F. virginiana* has been identified for its superior stress tolerance (waterlogged soil, cold, heat, and frost) and disease resistance (Hancock and Bringhurst 1979; Hancock 1999).

The possibility of genetically improving photosynthetic capacity of the cultivated strawberry has been suggested by previous studies that have evaluated the photosynthetic capacity of *F. chiloensis* and hybrids with *F. x ananassa.* This study indicated that photosynthetic rate can be increased through breeding with *F. chiloensis* (Hancock et al. 1989). When comparing two or more species, it is necessary to have a quantifiable understanding of the variability within species in order to determine if the desirable characteristic can be found in any genotype or if the genotype must be carefully selected for its potential. The objective of the current study is to develop an understanding of the variability in morphological and photosynthetic characteristics that may exist within the species *F. chiloensis* and *F. virginiana*.

Materials and Methods

Plant Material. Genotypes were selected based on inclusion in the 'supercore' which is a group of genotypes that have been identified as having potential for breeding purposes. Genotypes from a range of collection locations were utilized in order to capture as much variability in the species as possible. Unrooted runners were provided by the National Clonal Germplasm Respository (Corvallis, OR) of five genotypes each of *F. chiloensis* and *F. virginiana* (Table 3-1). Runners were rooted and propagated in the greenhouse to achieve adequate numbers. One year-old rooted runners were transplanted on 5 May 2006 to 4.4 liter pots filled with 1:1 mix of sand:Metro Mix® (Scott's Co., Marysville, Ohio). The plants were grown under natural conditions at the Cornell Orchards in Ithaca, New York, USA (Lat. 42.44 N, Long.76.50 W) for the summer and after dormancy, were placedin a cooler in order to meet chilling requirement.

Table 3-1 PI number, collection location, notes and indication of supercore status for all plant material used in the current study.

	PI Number	Genotype	Collection Location	Notes	Supercore
F. chiloensis	551445	'Lucidia'	California, USA		Yes
		'PigeonPt'			No
	551650	'PYB'	Yaquina Bay, Oregon, USA	Prolific runners; no fruit set	Yes
	612490	'Pacifica'	California, USA found in dunes	Female; short day; large; highest yield potential of any N. American clone; probably salt, drought, and low nutrient tolerant	Yes
	616585	'1203'	Chile	Small fruit	
F. virginiana	612496	'MN8688'	Alaska, USA	Partial hermaphrodite; representative of subsp. glauca from the upper northwest	Yes
	612495	'1697'	Montana, USA	Hermaphrodite; cyclic flowering; large, numerous fruit; probably cold winter hardy; found at 2255m elevation in Rocky Mtns.	Yes
	612494	'1696'	South Dakota, USA	Female; cyclic flowering; probably winter hardy; found at 1500m elevation in Rocky Mtns.	Yes
	612492	'1694'	Ontario, Canada	Partial hermaphrodite; weak; cyclic flowering	Yes
	612495	'LH50'	Montana, USA	Hermaphrodite; cyclic flowering; large, numerous fruit; probably cold winter hardy; found at 2255m elevation in Rocky Mtns.	Yes

In February 2007, plants were removed from the cooler and 12 crowns of each genotype were divided from their mother plants and potted in standard 4.4 L pots with one crown per pot. On 3 March 2007 the plants were arranged on greenhouse benches in four randomized blocks, each containing three repetitions per genotype. The plants were spaced with approximately 6 inches between pots to prevent mutual shading. The temperature of the greenhouse was set at 22/16 ^{O}C day/night temperature. The plants were placed under high pressure sodium supplemental lighting with an approximate

light level of 400-600 μmoles $m^{-2}s^{-1}$ with a day length of 16 hours (8 hour dark period). Plants were watered twice daily and fertilized every ten days with 15-5-15 fertilizer. Flowers were continuously removed throughout the experiment. The plants were sprayed for spider mite infestation twice.

Photosynthetic Data. Conditions were constant for all photosynthetic measurements; ambient CO_2 (360μmol mol^{-1}), temperature and water vapor pressure were maintained at 25°C ± 1°C and 2.3 kPa, respectively. Light adapted measurements of CO_2 assimilation (A_{CO2}) were made once a week on a recently expanded leaf between 1000-1400 hours. Light response curves were completed for 3 genotypes from each species. Three curves were run for each genotype for a total of 18 curves. Plants were dark adapted in the greenhouse under black shade cloth for 30-50 minutes in order to obtain dark adapted gas exchange and fluorescence measurements. All gas exchange and fluorescence data were collected simultaneously using the LiCor 6400 (LiCor Inc, Lincoln, NB) open gas exchange system with a fluorescence head. Light levels ranged from 0-2000 μmoles $m^{-2}s^{-1}$. Light response curves were analyzed using SAS 9.1 Proc NLIN with the Marquardt Method. Single leaf measurements were also taken on a recently fully expanded leaf of all the plants in the study. All other statistical analysis was conducted using JMP (SAS Institute Inc, Cary, NC).

Calvin Cycle Enzymes. Ten 1-cm^2 leaf disks were collected from three plants in each experimental unit at noon under full light and immediately placed in liquid N_2. Material was stored at -80°C until the time of the assay. Rubisco, NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC1.2.1.12) and fructose-1,6-bisphosphotase (FBPase, EC3.1.3.11) were extracted according to protocol described by Chen and Cheng (2003). Rubisco, GAPDH and FBPase extractions were carried out using a total of three leaf disks. In a precooled mortar and pestle, the leaves were ground in 1.5mL of a buffer solution containing 50 mM Hepes-KOH, 10mM $MgCl_2$,

2mM ethylenediaminetetraacetic acid (EDTA), 10mM DTT, 1%(v/v) Triton X-100, 5% (w/v) bovine serum albumin (BSA), 10% (v/v) glycerol, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged for 5 mins at 13,000rpm and the supernatant was used immediately for spectrophotometric analysis of enzyme activity.

Growth Analysis. Destructive growth analysis was conducted at the end of the study. Leaf area, leaf, runner, crown and root fresh weight and dry weight (DW) were collected by drying in an oven at 25^{o}C to a constant weight. Only dry weight data are reported in this study. Root: shoot ratio (R:S) and specific leaf weight (SLW) were calculated based on dry weights. Statistical analysis was carried out in JMP (SAS Institute Inc, NC).

Results

Growth Analysis. There were significant differences between the two species in leaf area, crown, root, root:shoot ratio (R:S) and specific leaf weight (SLW). *F. chiloensis* has more consistent crown, root and runner DW, with only leaf DW showing significant variation among genotypes. *F. virginiana* showed significant variation among genotypes in carbon allocation to all plant components measured (Table 3-2). The two species did not show any within species variability of R:S ratio; however, it was almost double in *F. virginiana* compared to *F. chiloensis*. *F. chiloensis* had significant variation in SLW within the species, and the mean for the species was double compared to *F. virginiana* which had no within species variability. (Table 3-2, Table 3-1).

Table 3-2. Growth analysis parameters for five genotypes of *F. chiloensis* and *F. Virginia*. Dry weights (DW) are expressed in gm/plant.

Genotype	Leaf Area (cm^2)	Leaf DW (gm/plant)	Runner (gm/plant)	Crown (gm/plant)	Root (gm/plant)	Total (gm/plant)	R:S	SLW (g/cm)	LAR
F. chiloensis									
1203	938ab	17.0abc	6.3a	5.5a	5.7a	34.4bc	0.48a	0.035c	27.2a
Lucidia	845ab	14.4bc	10.6a	6.3a	6.4a	37.6abc	0.51a	0.038bc	22.5ab
Pacifica	1226a	19.8ab	8.9a	8.1a	7.7a	44.5ab	0.56a	0.042ab	27.6a
PigeonPt	1149ab	21.5a	9.4a	8.1a	8.2a	47.2a	0.57a	0.043a	24.3ab
PYB	617b	12.0c	8.3a	4.0a	4.8a	29.1c	0.42a	0.030d	21.2b
Mean	***955B***	***16.9A***	***8.68A***	***6.4B***	***6.5B***	***38.5B***	***0.51B***	***0.038A***	***24.8B***
F. virginiana									
1694	1476a	13.3bc	1.6b	5.8a	7.9b	28.4c	0.91a	0.019a	51.97a
1696	1684a	12.9bc	6.3ab	8.1a	15.2ab	42.4bc	1.21a	0.021a	39.7b
1697	1836a	21.4a	8.8a	9.6a	20.4a	60.1a	0.98a	0.021a	30.5c
LH50	1722a	17.6ab	10.3a	10.5a	13.3ab	51.7ab	0.86a	0.019a	33.3bc
MN8066	1382a	11.4c	8.1a	10.3a	12.3ab	42.1bc	1.18a	0.019a	32.8c
Mean	***1607A***	***15.3A***	***7.0A***	***8.84A***	***13.8A***	***44.9A***	***1.02A***	***0.020B***	***35.8A***

Means with same letter not significantly different according to Tukey's HSD. Species means are compared with capital letter, within species means compared with lower case letters.

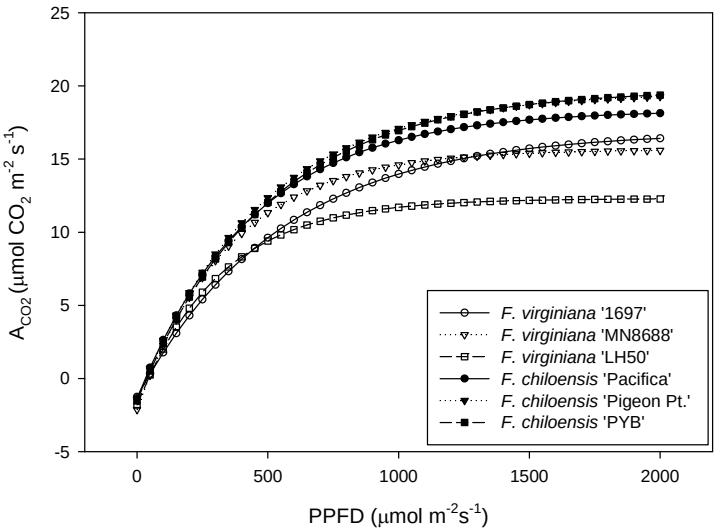

Figure 3-1. Light response curves of three sub-species of *F. virginiana* (open markers) and *F. chiloensis* (closed markers)

Photosynthetic Data. Light response curves of three *F. chiloensis* genotypes were not significantly different at any light level (Figure 3-1). However, *F. virginiana* showed significant variation at lower light levels between *F. virginiana* 'MN8688' and '1697' which had lower rates at light levels below 525 µmol PPFD $m^{-2}s^{-1}$. However, at light levels above 1100 µmol PPFD $m^{-2}s^{-1}$, both of these genotypes had A_{CO2} rates that were higher compared to 'LH50' which had the lowest overall rates of A_{CO2} under saturating light conditions (Figure 3-1).

Midday single leaf measurements of A_{CO2} and g_s showed a significant difference between species means; however, there was little variability in A_{CO2} rates and stomatal conductance (g_s) within species (Table 3-3)

Table 3-3. Midday photosynthetic rate (A_{CO2}) and stomatal conductance (g_s) of 5 genotypes of *F. chiloensis* and *F. virginiana.*

	A_{CO2} (μmol $m^{-2}s^{-1}$)	Cond (mol m^{-2} s^{-1})	Fv'/Fm'
F. chiloensis			
1203	24.7 a	0.41 a	0.71 a
lucidia	25.3 a	0.40 ab	0.53 a
Pacifica	22.5 a	0.37 ab	0.58 a
Pigeon Pt	22.7 a	0.34 ab	0.60 a
PYB	19.7 a	0.24 b	0.57 a
Mean	**22.4 A**	**0.34 A**	**0.59 A**
F. virginiana			
1694	9.8 b	0.11 a	0.44 a
1696	12.5 ab	0.15 a	0.45 a
1697	15.0 a	0.26 a	0.51 a
LH50	11.1 ab	0.14 a	0.43 a
MN8688	11.5 ab	0.20 a	0.45 a
Mean	**11.9 B**	**0.17 B**	**0.45 B**

Within species means with the same lower case letter or Mean with same upercase letter are not significantly different according to Tukey's HSD

There was no within species variability for light adapted Fv'/Fm' values, at any light levels (Figure 3-3). Under light conditions that were higher than 1200 μmol PPFD $m^{-2}s^{-1}$, *F. chiloensis* had higher mean Fv'/Fm' compared to *F. virginiana* ingle leaf measurements of all the included genotypes confirm the consistently higher values of Fv'/Fm' observed in *F. chiloensis* (Table 3-3).

Rubisco activity is consistently higher in *F. chiloensis* on both a leaf area and mass basis (Figure 3-3). All genotypes of *F. virginiana* were similar; however, *F. chiloensis* 'Pigeon Pt.' had significantly higher Rubsico activity compared to the genotype 'lucidia' (Figure 3-3A). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was significantly higher in *F. chiloensis* on a leaf area basis; however, when expressed on a leaf mass basis, there was no significant difference. There was no within species variability observed in GAPDH activity (Figure 3-3B). There was no

difference observed in fructose bisphosphotase (FBPase) activity between the two species nor was there within species variability on either a leaf area or leaf mass basis (Figure 3-3C). Phosphoribulose kinase (PRK) activity was higher in *F. chiloensis* compared to *F. virginiana* on a leaf area basis; however, this was not the case when expressed on a mass basis. Within species variability was observed in *F. chiloensis* on a leaf mass basis and in *F. virginiana*, genotype 'MN8688' had higher rates compared to '1694' on a leaf area basis (Figure 3-3)

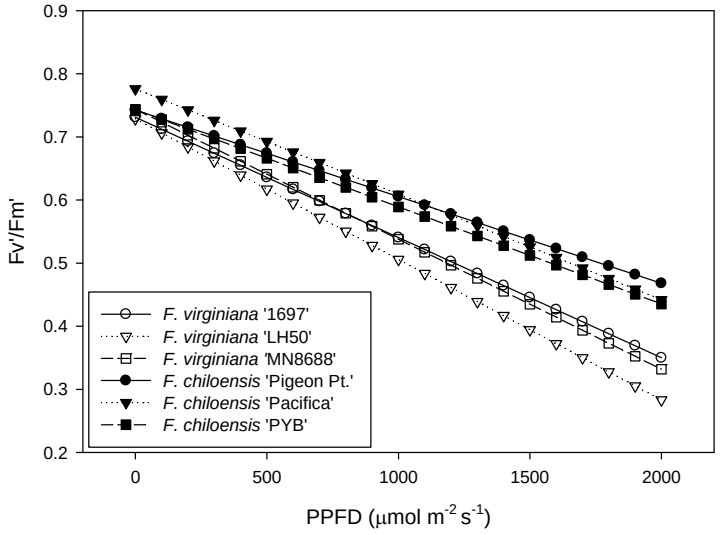

Figure 3-2. Light response curves of light adapted quantum efficiency of Fv'/Fm' of three *F. virginiana* genotypes (open markers) and three *F. chiloensis* genotypes (closed markers).

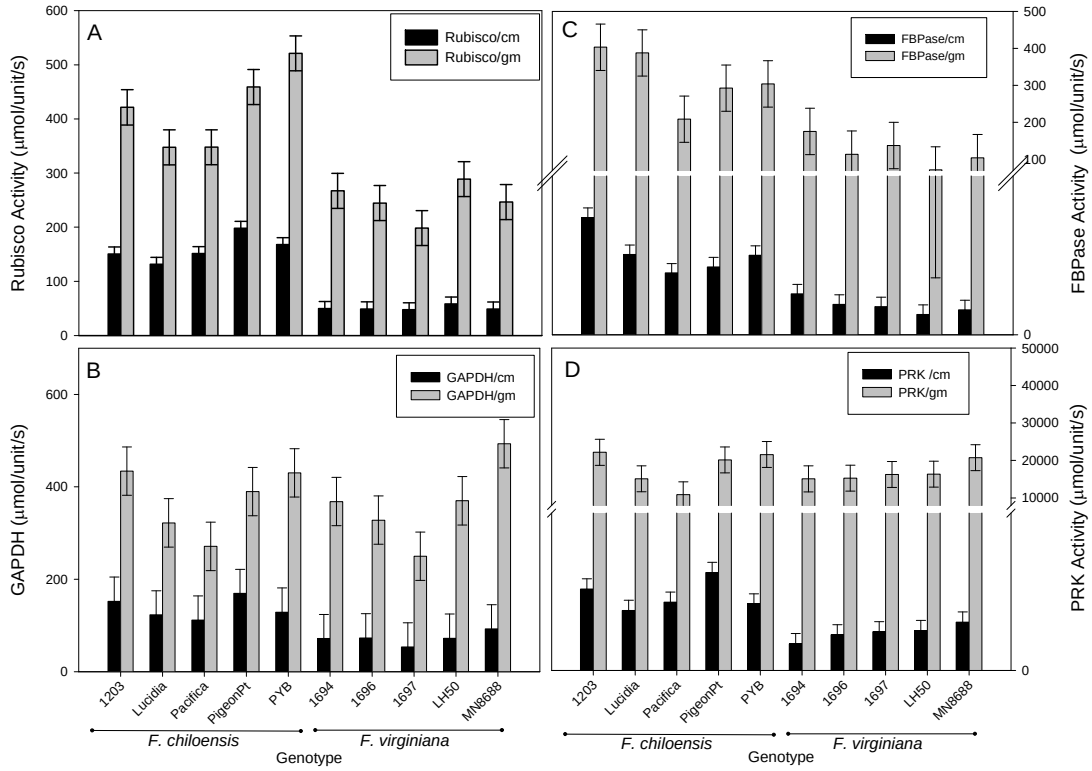

Figure 3-3 Enzyme activity of Rubsico (A), GAPDH (B), FBPase (C) and PRK (D) on a leaf area and leaf mass basis of five genotypes of *F. chiloensis* and *F. virginiana.*

Discussion

Both species had within species variability in leaf dry weight (DW). *F. chiloensis* also displayed variability between genotypes in leaf area and SLW (Table 3-2). Previous studies using principal component analysis found that leaf size and shape account for the greatest portion in morphological variability between the canopies of *F. virginiana* and *F. chiloensis* (Harrison et al. 1997). In the current study, the within species variability was greater than between species for leaf dry weight; however, *F. chiloensis* had almost half the leaf area compared to *F. virginiana*, resulting in almost twice the specific leaf weight (SLW) in *F. chiloensis.* This is a typical characteristic of *F. chiloensis* that has consistently been observed (Darrow 1966; Hancock and Bringhurst 1979; Hancock et al. 1999; Hancock 1999). Although there is some

variation between genotypes within the species, all gentoypes are still close to double the SLW compared with *F. virginiana* which had consistent SLW across all genotypes (Table 3-2).

Leaf and runner components showed more variability between genotypes within a species, the crown and root DW had little within species variability but significant differences between the species. The mean crown and root DW of *F. virginiana* were 38% and 112% greater in *F. virginiana,* respectively (Table 3-2). This is reflected in the R:S ratio which was twice as high in *F. virginiana* compared to *F. chiloensis.* The R:S ratio can be an indication of source-sink ratios, as the crown and roots are typical sinks to the plant and the leaves are sources.

Other characteristics that have been correlated with A_{CO2} are SLW and LAR (Field and Mooney 1986; Reich et al. 1998b). Typically, a low SLW, which is often associated with shade species, tends to result in lower rates of A_{CO2}. This was observed in the current study as well. *F. virginiana*, which is native to forest habitats, has both a lower SLW (Table 3-2) and consistently lower A_{CO2} (Figure 3-1). Leaf area ratio (LAR) has been correlated with A_{C02} rate (Buttery and Buzzell 1972) where a high LAR results in decreased A_{CO2} due to increased source capacity and subsequent suppression of photosynthetic enzymes (Buttery and Buzzell 1972; Poorter and Evans 1998). *F. virginiana* had significantly higher LAR ratio and had consistently lower A_{CO2} rates compared to *F. chiloensis* due to the much higher leaf area of *F. virginiana* (Figure 3-2, Table 3-3). Although both LAR and SLW have been linked to A_{CO2} in other species, it is difficult to determine how much of the variability observed within and between species photosynthetic rates are due to source-sink balances due to the complexity of the regulation system that affects photosynthetic parameters. The results of this study indicate that although there is within species variability in some of the growth variables, the plant structure of the two species is consistent. *F. virginiana* is a larger plant with a

large root system and crown, the canopy is dense, with thin leaves. *F. chiloensis* is a smaller plant with thick glossy leaves and a small root system and crown. If a species is being utilized in breeding for a particular plant structure, the desirable structure can likely be found in any genotype due to the consistency of these characteristics.

The Fv'/Fm' data gives an indication of the efficiency of the open PSII reaction centers under illuminated conditions. Consistent with the gas exchange data, *F. chiloensis* had consistently higher Fv'/Fm' at all light levels and there was no significant difference between the *F. chiloensis* genotypes (Figure 3-2, Table 3-3). The fluorescence data suggest that *F. chiloensis* is able to harvest and utilize more light energy for a given leaf area, which would be expected as it is native to high light conditions (Wilhelm and Sagen 1974) and that the within species differences in photosynthetic rate observed are not due to differences in the quantum efficiency, but may be due to either source-sink relations or of the ability of the plant to regenerate RuBP (Farquhar et al. 1980).

The regeneration of RuBP is a possible limitation to photosynthesis and differences in enzyme activity may suggest a biochemical basis for the differences between the two progenitor species. Rubisco is the enzyme active in the carboxylation process and *F. chiloensis* had significantly higher activity compared to *F. virginiana* (Figure 3-3A). *F. chiloensis* had some variability in genotypes within the species whereas *F. virginiana* showed no variability across genotypes. There was no difference in the activity of GAPD between species means, but there was a great deal of variability between genotypes (Figure 3-3B). Although there is no significant difference in FBPase activity between the species means, there is a trend of higher rates in *F. chiloensis* plants compared to *F. virginiana* (Figure 3-3C). FBPase is suppressed in conditions of high sugar accumulation which would occur under sink-limited conditions (Krapp et al. 1993b) which would be suggested by the higher LAR observed in the *F.*

virginiana species and may be contributing to the lower rates of enzyme activity. PRK showed no significant difference on a dry weight basis between species but *F. chiloensis* had higher activity on a leaf area basis.(Figure 3-3D). The results of this study show that Rubisco is the only photosynthetic enzyme that consistently had higher rates of activity in *F. chiloensis* on both an area and dry weight basis.

It should be noted that the activity of many enzymes in this study are lower than what would be observed in plants grown out in full sunlight. The lower light levels of the greenhouse during the winter could result in the activity of the enzymes being suppressed and therefore not operating at the highest levels where differences between species may be greater.

Summary

The differences in photosynthetic capacity that have been observed between *F. chiloensis* and *F. virginiana* in previous studies appear to be consistent across the genotypes of the two species that were evaluated in this study and are also consistent with sun and shade-adapted species. *F. chiloensis,* the sun-adapted species, had consistently higher whereas *F. virginiana,* the shade adapted species was a larger plant with thin, large leaves and lower photosynthetic capacity. These sun/shade characteristics are exhibited when the plants are grown in the same environment.

The genotypes within the two species displayed similar growth characteristics that are associated with the species, despite some minor within species variability. The results of this study indicate that the selection of wild species to exploit a morphological characteristic of a species may not have to be concerned with utilizing a particular species. However, if the interest is in the photosynthetic activity and in particular, in the activity of photosynthetic enzymes, then multiple genotypes within a species should be evaluated due to the variability observed.

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CHAPTER 4

RETROSPECTIVE OF ANALYSIS OF CULTIVAR DEVELOPMENT IN THE NORTHEASTERN UNITED STATES DURING THE 20TH CENTURY

Introduction

Development of the Cultivated Strawberry. Evidence of strawberry cultivation can be found in literature dating back as far as the sixth century. However, the history of the modern strawberry is fairly recent and begins in the 16th and 17th century with the exploration of North and South America. In the 17th century, French and British explorers brought back *Fragaria virginiana* L. to Europe where Jean Robin, a botanist to King Henri IV of France cultivated the plant in the royal garden. Although native strawberries existed in Europe, they were soft and small. Over time, selections were made and cultivars of *F. virginiana* began to appear throughout Europe. In the early 18th century, *F. chiloensis* L. was introduced to Europe from Chile by a French spy, Captain Amédée Fraziér, a French engineer and mathematician to Louis XIV of France (Darrow 1966; Wilhelm and Sagen 1974). *F. chiloensis* did not gain as much popularity as *F. virginiana* in Europe due to sterility problems, but was of continued interest due to the large fruit size. By the mid-1700s, a new type of strawberry that began to appear in Europe that was far superior to both *F. virginiana* and *F. chiloensis.* After much speculation as to its origin, it was correctly identified by Antoine Duchesne in 1766 as a hybrid between the two species; *F. virginiana* and *F. chiloensis* and was named *F. x ananassa* Duch. due to the pineapple aroma that was associated with it. The chance appearance of this hybrid marked the beginning of the development of the modern strawberry, *F. x ananassa*.

The first systematic breeding of strawberries began in England in 1817 by Thomas A. Knight, an associate of Charles Darwin, who developed important cultivars such as 'Elton' and 'Downton' (Darrow 1966). At about the same time, Michael Keen developed 'Keen's Seedling' which became the dominant cultivar in Europe for close to a century and is in the background of many of today's modern cultivars (Hancock 1999).

The early American strawberry varieties were selections of the small fruited species *F. virginiana*, known as the Scarlet strawberry, and at least 30 varieties were available by 1820 (Jones 1976). The introduction of the much larger fruited *F. x ananassa* varieties (Pineapple strawberry) from Europe quickly became the dominant strawberry grown particularly with the release of 'Hovey', the first American *F. x ananassa* cultivar released by Charles Hovey of Cambridge, MA in 1836 (Hedrick 1925). The success of this cultivar also stimulated amateur breeders to engage in the pursuit of the larger strawberry and by the end of the 19th century several important cultivars had been developed including 'Cresent' and 'Marshall'. New, improved cultivars continued to be released in the early part of the 20th century such as; 'Dunlap', 'Klondike', 'Howard 17' and 'Aberdeen' all of which played an important role in the growing American strawberry industry (Darrow 1966). During the early stages of cultivar development, the improvement of size was a priority, but increasingly important were characters such as disease resistance and fruit characteristics (i.e. flavor, firmness, color) all of which remain a priority today. By the early 1900s, breeders were focused on developing cultivars for a particular region due to the specific conditions of the area; this regionality of cultivar development continues today, with the exception of California varieties which are grown throughout Europe.

In the northeastern U.S., strawberries are produced in a perennial system, and therefore, disease resistance has been a primary focus of breeders who have

successfully developed several cultivars with resistance to many of the common diseases in the region. As a result, breeding for high yield has been more challenging compared to breeders developing cultivars for an annual system which is primarily focused on fruit size, firmness and yield. The objectives of this study were to evaluate some of the top cultivars that have been released in the northeastern U.S. during the past century for yield, fruit quality and photosynthetic performance. By identifying possible limitations to yield improvement, new strategies may be suggested to overcome the apparent plateau in cultivar development in the northeastern U.S.

Materials and Methods

Plant Material. Twenty strawberry (*F. x anananssa*) cultivars (Table 4-1) and three genotypes of each of the progenitor species, *F. chiloensis* and *F. virginiana*, were included in the trial. Plants were obtained from commercial growers or from the National Germplasm Repository (Corvallis, Ore). Runners were established in a mist bed before transplanting to 6" pots filled with 1:2:1 (perlite:peat:vermiculite) and place in the greenhouse under supplemental high pressure sodium lights.

Greenhouse Trial. Plants were arranged on benches under high pressure sodium lights. Average light levels were 500 μmol $m^{-2}s^{-1}$ with day/night temperature of 23/18^{o}C. Plants were deflowered and derunnered during the greenhouse trial. The trial was set up as a randomized complete block design with four blocks and 6 plants in each experimental unit.

Field Trial. Plants from the greenhouse trial were cold acclimated and then placed in a cooler for 6 weeks to obtain the required chilling period, then in May 2006 was planted in a matted row field planting in Ithaca, NY (Lat. 40.44N Long. 76.5W).

The trial was set up in a randomized complete block design with four blocks and six plants in each experimental unit.

Photosynthetic Data. Gas exchange and chlorophyll fluorescence data were collected using the LiCor 6400 (Li-Cor, Lincoln, Neb.). Data were collected on a recently expanded leaf between the hours of 1000 and 1400. Light levels in the chamber were set to match ambient conditions for each trial; for the greenhouse trial light was set at 500μmol PAR $m^{-2}s^{-1}$, and the field trial was set at 1500 μmol PAR $m^{-2}s^{-1}$.

Table 4-1. Cultivar name, date of release, parentage and breeding program of cultivars included in the study. Cultivar names followed by * indicate selections used in the carotenoid study.

Cultivar Name	Release Date	Parentage	Breeding Program Location
Royal Sovereign*	1891	Noble' X 'King of the Earliest'	UK
Marshall	1893	American selection of cultivated strawberry	Massachusetts
Dunlap*	1900	probably 'Cresent' x 'Cumberland Triumph'	Illinois
Klondike	1901	Pickerproof' x 'Hoffman'	Louisiana
Aberdeen*	1924	Likely 'Late Stevens' x 'Chesapeake'	New Jersey
Blakemore	1929	Missionary' x 'Howard 17'	Maryland
Fairfax*	1933	Royal Sovereign x Howard 17	Maryland
Sparkle*	1942	Fairfax x Aberdeen	New Jersey
Jerseybelle*	1955	NJ953 x NJ925	New Jersey
Surecrop	1956	Fairland x USMD1972	Maryland
Raritan*	1968	Redglow' x 'Jerseybelle'	New Jersey
Guardian*	1969	NC1768 x 'Surecrop'	Maryland
Earliglow	1975	MDUS 2359 ('Fairland' x 'Midland') X	New York
Honeoye*	1979	Vibrant' x 'Holiday'	New York
Allstar	1981	US4419x (NCUS1768 x 'Surecrop')	Maryland
Jewel*	1985	NY 1221 x 'Holiday'	New York
Northeaster*	1993	MDUS 4380 x 'Holiday'	New York
Cabot	1999	K87-5 x K86-19	Nova Scotia
L'Amour*	2003	(MDUS5252 x 'Etna') x 'Cavendish'	New York
Ovation	2003	Lateglow' x 'Etna'	Maryland

Fruit Quality. Subsamples of 10 king berries from each plot were collected during the fruiting period and used to measure berry firmness using a Wagner Force Rive FDV-30 force gauge (Wagner Instruments, Greenwich, CT) with a 15 mm tip. Twenty g subsamples of fruit were collected during the fruiting period to measure soluble solids with a digital refractometer (ATAGO USA Inc. Bellevue, WA).

Anthocyanins and Phenolics. Whole fruit were extracted in 80% methanol and 0.2% folic acid buffer solution. Total anthocyanins were measured using the pH differential method and total phenolics were measured using the Folin-Ciocalteu procedure (Singleton et al. 1999). All samples were analyzed in triplicate.

Carotenoid/Chlorophyll Data. A subset from the greenhouse trial of 12 cultivars and one genotype of *F.chiloensis* and *F.virginiana* (Table 4-1) were used for pigment analysis. Leaf discs were collected between 11:00-13:00 from the center leaflet of the last fully expanded leaf, weighed and placed in liquid N2. Pigments were extracted under low light from 50-100 mg of leaf tissue. Tetrahydrofuran and methanol (MeOH) were used as solvents with butylated hydroxytoluene as an antioxidant. Pigments were then transferred to ether via phase partitioning, the ether removed in vacuo, and the extract re-suspended in MeOH/methyl t-butyl ether (1:1). HPLC was performed with an ASI-100 automated sample injector linked to a P680 HPLC pump and a PDA-100 photodiode array detector (Dionex Corp., Sunneyvale, CA). Pigments were eluted from a C30 reverse-phase column (Waters Inc., Milford, MA) via a linear solvent gradient (20 min) consisting of ammonium-acetate in MeOH (1 $g.L^{-1}$) and methyl t-butyl ether.

Growth Analysis. Flower counts and date of first flower were recorded on 3 plants in every plot during the spring. After harvest, three plants per plot were dug up

from the field and used for destructive growth analysis. Plants were washed and separated into individual growth components (leaves, crown, roots) then dried at 75°C for 3 days before recording dry weights. Leaf area was measured on all plants before drying leaves.

Results

Greenhouse Trial. There was no observed difference in the rate of photosynthesis on a leaf area basis between the cultivars evaluated. The mean of the cultivars (14.1 μmol CO_2 m^{-2} s^{-1}) was intermediate to the photosynthetic rates of the progenitor species; *F. virginiana* (11.6 μmol CO_2 m^{-2} s^{-1}) and *F. chiloensis* (17.8 μmol CO_2 m^{-2} s^{-1}). There was also no significant change in stomatal conductance (g_s) (Figure 4-1 A,B).

Fv'/Fm' is a measure of the efficiency of the open PSII and ΦPSII is the overall efficiency of PSII. Regression analysis of the fluorescence data indicate that there has been a decrease of 5% in the Fv'/Fm' over the last century. Initial mean Fv'/Fm' for the early cultivars (0.68) was intermediate to the two progenitor species; *F. chiloensis* (0.72) and *F. virginiana* (0.62) (Figure 4-1C). ΦPSII has decreased by 10% during the last century. Again, initial rates were intermediate to the progenitor species; *F. chiloensis* (0.61) and *F. virginiana* (0.50) and have become more similar to *F. virginiana* (Figure 4-1D).

One of the components of non-photochemical quenching, heat dissipation, is carried out by zeaxanthin in the PSII antennae complex (Horton and Ruban 2005). And other pigments also participate in photosynthetic processes. α–carotene, β–carotene, zeaxanthin and violaxanthin levels of the cultivars have remained constant over time and are intermediate to the progenitor species (Table 4-2). The total amount of

chlorophyll (a+b) on a leaf area basis has remained intermediate to the progenitor species (Table 4-2).

Field Trial. Photosynthetic data were collected at three phenological stages; flowering (11 May), peak fruiting (15 June) and late fruiting (31 June). The highest rates of $A_{CO2,}$ g_s and ΦPSII were observed during the peak fruiting stage. Although regressions were not statistically significant, with the exception of g_s during peak fruiting, there was a trend of decreasing A_{CO2}, $g_{s,}$ and ΦPSII with cultivars released over time and Fv'/Fm' has remained unchanged (Figure 4-2).

Total, marketable and unmarketable yield were highly variable across cultivars; however, there were no significant trends over the last century. The highest marketable yields were observed in cultivars released in the 1970s and 1980s (Figure 4-3). Although there were no significant trends in yield, average berry weight of both king berries and lower order berries (2^o, 3^o and 4^o) have increased over time (Figure 4-4) whereas average berry number has decreased (Figure 4-5).

There has been a significant increase in fruit firmness during the past century with current cultivars being almost 50% more firm compared to those from the turn of the century (Figure 4-6A). Average percent soluble solids has decreased over time from approximately 8.7% to 7.6% (Figure 4-6B).

Anthocyanin content (mg/g fruit fresh weight) varied almost three fold from the lowest cultivar Mme.Moutot (25mg/100gFW) to the highest in the cultivar Northeaster (72mg/100g fresh weight (FW)). Phenolic content also varied between cultivars with at low of 224mg/100g FW observed in 'Cabot' to a high of 409mg/100g FW in Aberdeen (Table 4-3). There were no observable trends over time in either anthocyanin content or phenolic content.

Flower counts taken in the spring indicate that the average number of flowers per plant has decreased by more than half over the last century (Figure 4-7). Plants have increased in total dry matter accumulation, primarily due to increased crown dry weight and a slight increase in root dry weight (Figure 4-8). There was no significant change in leaf dry weight or leaf area observed.

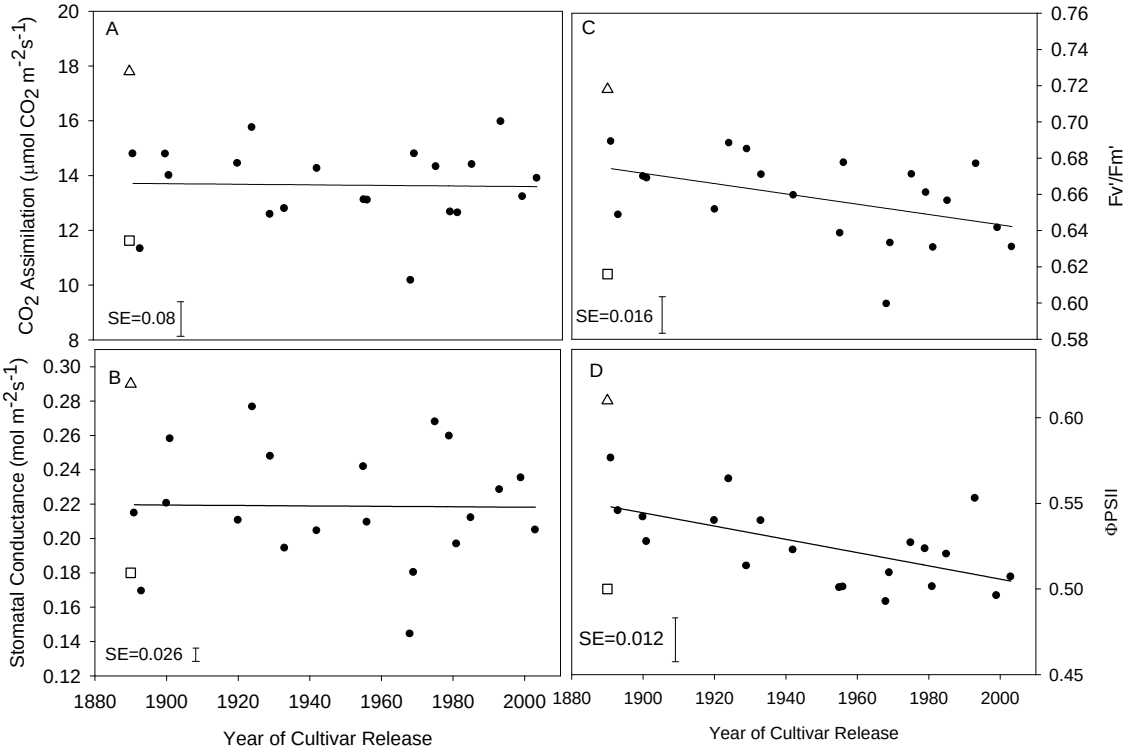

Figure 4-1 Photosynthetic characteristics of greenhouse grown cultivars released in the northeastern U.S. over the last one hundred years (mean of 6 points for each cultivar) and the progenitor species; *F. chiloensis* (open triangle) and *F. virginana* (open square) with regression of cultivars over date of release. CO_2 assimilation (A); stomatal conductance (B); Fv'/Fm' (C); ΦPSII (D).
Regression equations: Fv'/Fm' = 1.21 - 0.00028*Year (R^2=0.15, P=0.0019); PhiPSII = 1.23 - 0.00036*Year (R^2=0.15, P=0.0024).

Table 4-2. Leaf carotenoid content of cultivars and species grown in greenhouse**.**

Cultivar	Release Date	Chl A (g/cm^2)	Chl B (g/cm^2)	Chl A:B	α-carotene (mg/m^2)	β-carotene (mg/m^2)	Zeaxanthin (mg/m^2)	Violaxanthin (mg/m^2)	Leutin* (mg/m^2)
Royal Sovereign	1890	44.8	12.4	3.6	87.8	1250	88.8	56.3	1136
Marshall	1893	39.9	10.7	3.6	79.5	1082	65.1	33.3	1090
Dunlap	1900	42.9	12.1	3.6	75.1	1154	63.7	45.8	1101
Aberdeen	1924	35.1	9.4	3.4	79.0	1059	81.8	44.0	1090
Fairfax	1933	48.6	14.1	3.5	91.5	1287	40.2	26.5	1366
Sparkle	1942	42.8	12.2	3.6	87.3	1215	52.9	36.4	1224
Jerseybelle	1955	46.3	13.6	3.6	102.2	1297	52.9	37.5	1400
Raritan	1968	44.9	12.2	3.6	81.7	1224	79.1	47.3	1252
Honeoye	1979	49.5	13.6	3.7	98.5	1356	64.7	54.0	1467
Jewel	1985	39.7	11.6	3.3	77.4	1126	78.7	47.0	1267
Northeaster	1993	50.3	15.1	3.0	96.2	1373	54.4	35.2	1548
L'amour	2003	45.3	13.0	3.5	87.9	1226	72.8	42.5	1336
F. chiloensis		72.7	19.9	3.7	146.3	2113	68.6	44.0	1750
F. virginiana		31.3	9.3	2.8	57.3	853	111.8	66.7	884
Std Err		2.8	1.1	0.25	6.7	79.2	11.6	8	78.8
P-Value		0.0083*	0.0189*	NS	<0.0001	<0.0001	0.0092	NS	<0.0001

* Significant linear regression with increasing content over time (R^2=0.23, P<0.0001).

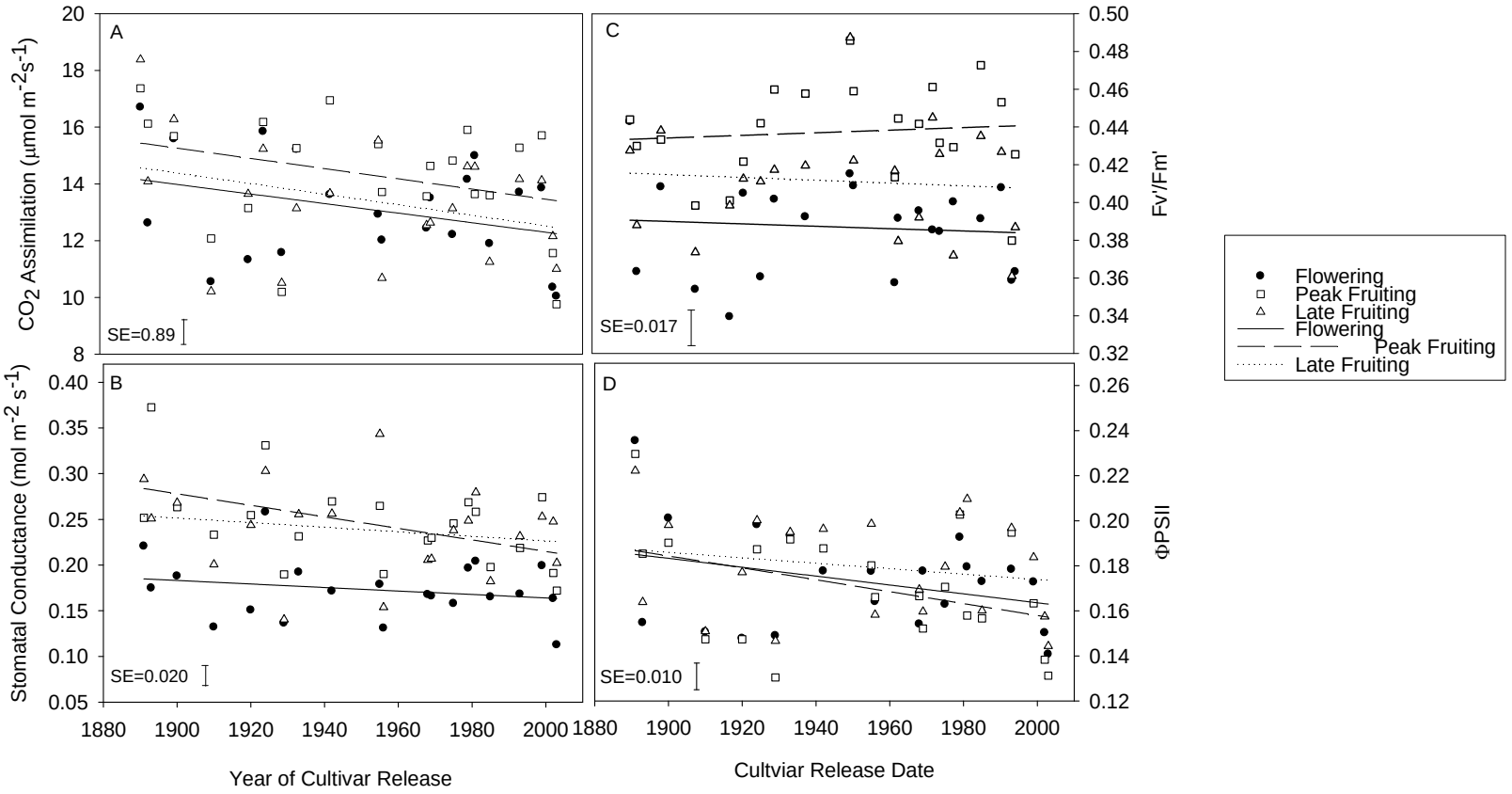

Figure 4-2. Maximum CO_2 assimilation rates (A), stomatal conductance (B), Fv'/Fm' (C) and photochemical quenching (D) measured on three dates: 1) Flowering (15 May 2008) 2) Peak Fruiting (11 June 2008) and 3) Late Fruiting (30 June 2008) of cultivars grown in a matted row field trial. Light level in chamber was 1500 µmol PFD $m^{-2}s^{-1}$.

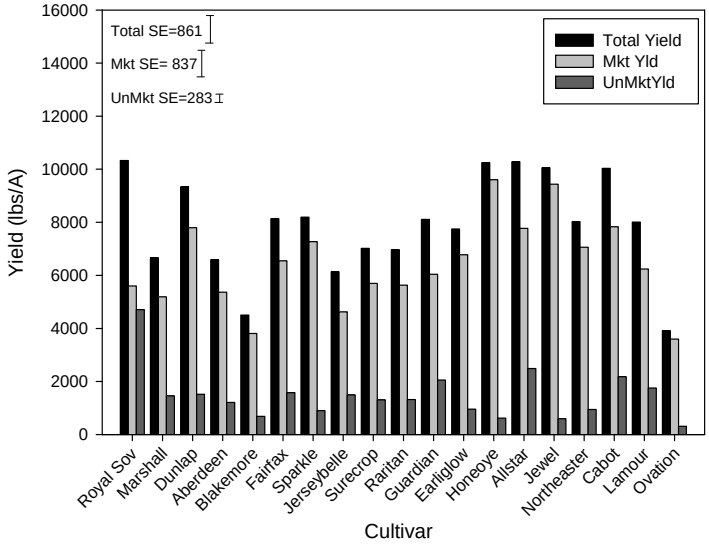

Figure 4-3. Total, marketable and unmarketable (5g or less or misshapen fruit) of 20 cultivars released over the last century. Cultivars listed in order of release date.

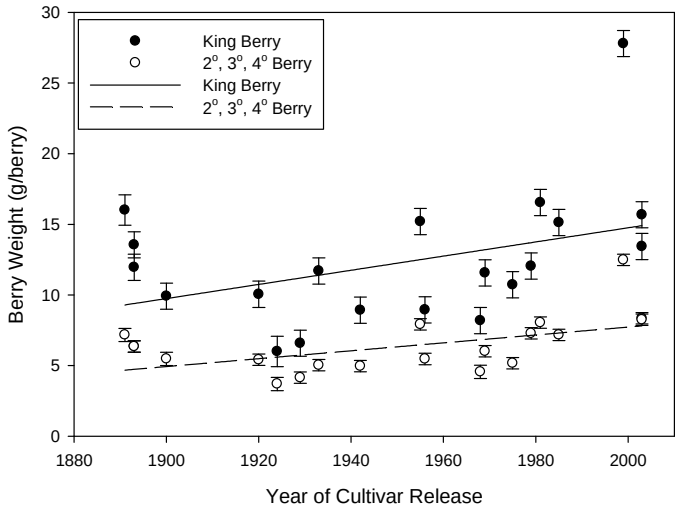

Figure 4-4. Average berry weight of King (1°) Berry, and 2°, 3°, 4° berries.
Regression equations: King berry, y=106.3+0.0606*Year (R^2=0.17, P<0.0001); 2°,3°,4° berry, y=-57.3+0.033*Year (R^2=0.29, P<0.0001)

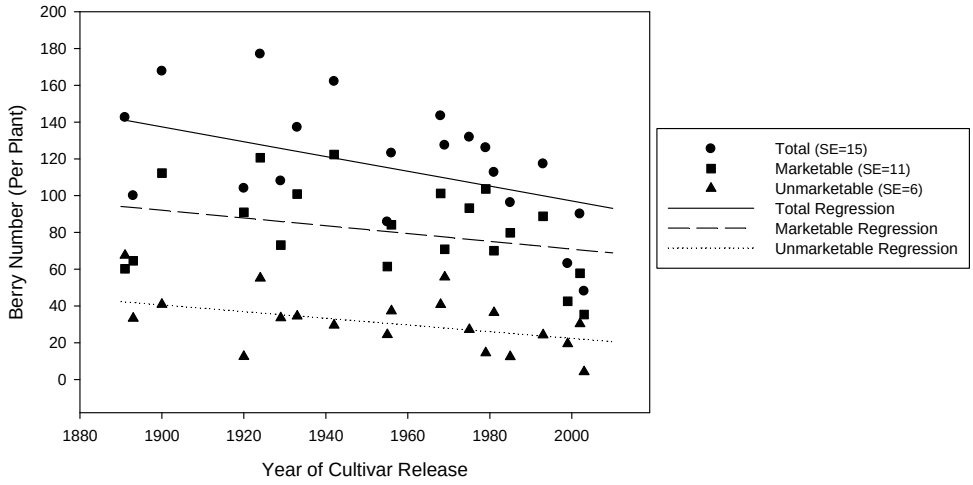

Figure 4-5. Average total, marketable and unmarketable (<5g or misshapen) berry number per plant grown in matted row field trial in Ithaca, NY.
Regression equations: Total, y= 902- 0.403*Year (R^2=0.14, P=0.0035); Marketable, = 493- 0.211*Year (R^2=0.05, P=0.0417); Unmarketable, y= 385 - 0.182*Year (R^2=0.14, P=0.0036)

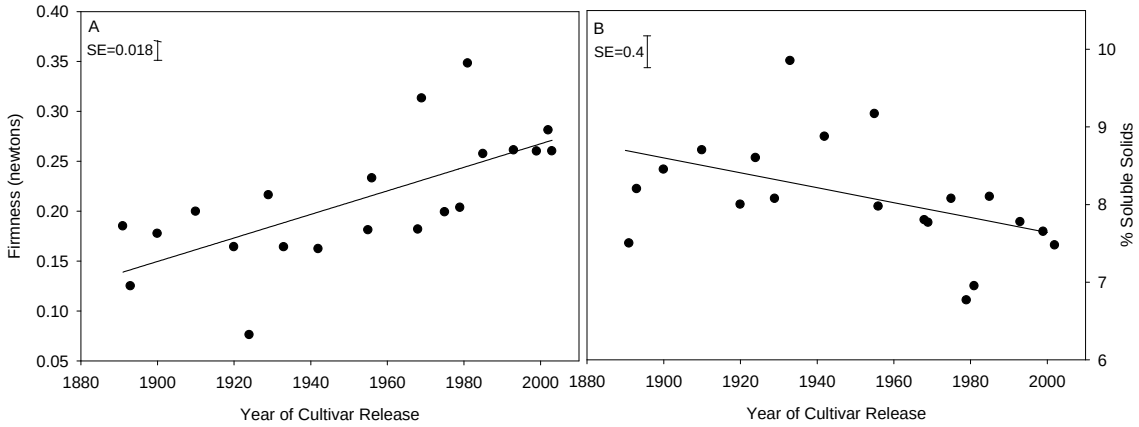

Figure 4-6. Berry firmness (newtons) and ^{o}Brix value of strawberry cultivars released over the last century. Points indicate mean of 20 berries.
Regression Equations: Firmness=-2.09 + 0.0012*Year (R^2=0.47; P=0.0006); Brix =26.84 - 0.0096*Year (R^2=0.11, P=0.0028)

Table 4-3. Anthocyanin and phenolic content (expressed in mg 3-phenyl-glucoside equivalents) of fruit grown in matted row field trial**.**

Cultivar	Release Date	Anthocyanins (mg/100g FW)	Phenolics (mg 3-P-glu/100g FW)
Royal Sovereign	1891	27	225
Marshall	1893	60	278
Dunlap	1900	53	318
Mme. Moutot	1920	25	343
Aberedeen	1924	39	409
Blakemore	1929	29	265
Fairfax	1933	45	253
Sparkle	1942	69	275
Jerseybelle	1955	66	271
Surecrop	1956	57	345
Raritan	1968	46	308
Guardian	1969	42	350
Earliglow	1975	59	380
Honeoye	1979	59	326
Allstar	1981	32	305
Jewel	1985	62	301
Northeaster	1993	72	317
Cabot	1999	52	224
L'Amour	2003	36	266
Ovation	2003	37	341
Std Err		3	24
P-value		<0.0001	<0.0001

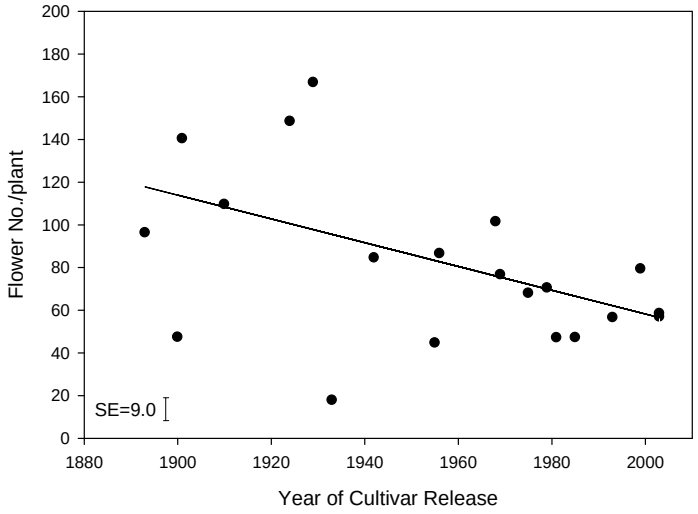

Figure 4-7. Number of flowers/plant on cultivars grown in field trial.
Regression equation: y = 1018- 0.48*Year (R^2=0.21, P=0.0431)

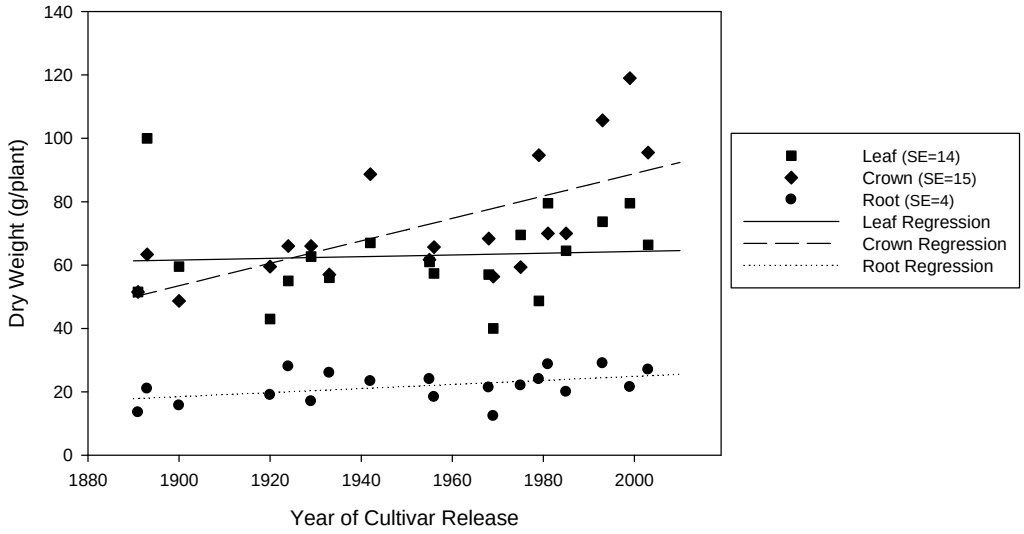

Figure 4-8. Leaf, crown and root dry weights per plant of strawberry cultivars grown in matted row field trial.
Regression equations: Leaf (NS); Crown= -619 + 0.35*Year (R^2=0.20, P=0.0005); Root= -103 + 0.064*Year (R^2=0.08, P=0.03).

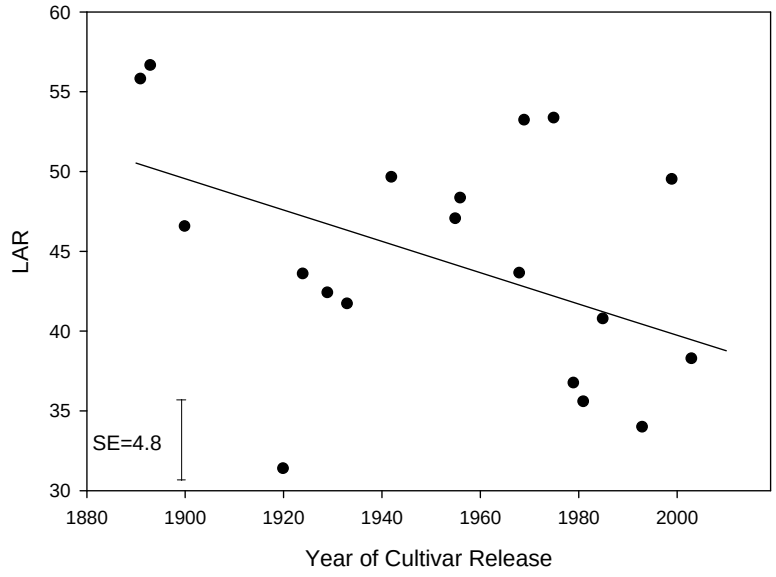

Figure 4-9 Leaf area ratio (LAR= leaf area/plant dry weight) of cultivars released in the northeastern U.S. during the 20th century.
Regression equations: LAR= 235.95- 0.098*Year (R^2=0.13, P=0.0135)

Discussion

During the last century, there have been hundreds of cultivars released in the northeastern United States with the goal of producing a higher yielding cultivar with superior fruit quality; however, the data from this study suggest that there have been limited increases in yield, particularly over the last four decades. The cultivars 'Honeoye' (1979), 'Allstar' (1981) and 'Jewel' (1985) had the highest marketable yields in this study (Figure 4-3) and have been three of the most planted cultivars in the northeastern U.S. since their introduction. 'Jewel' in particular is perhaps the most widely grown cultivar in the Northeast, almost 25 years after its introduction. These results are consistent with census data collected for New York which show no significant increase in yields between 1970 and 1994 (Figure 4-10). Although yields have increased in the U.S., the majority of those increases have been realized in California and Florida (Bertelson 1995).

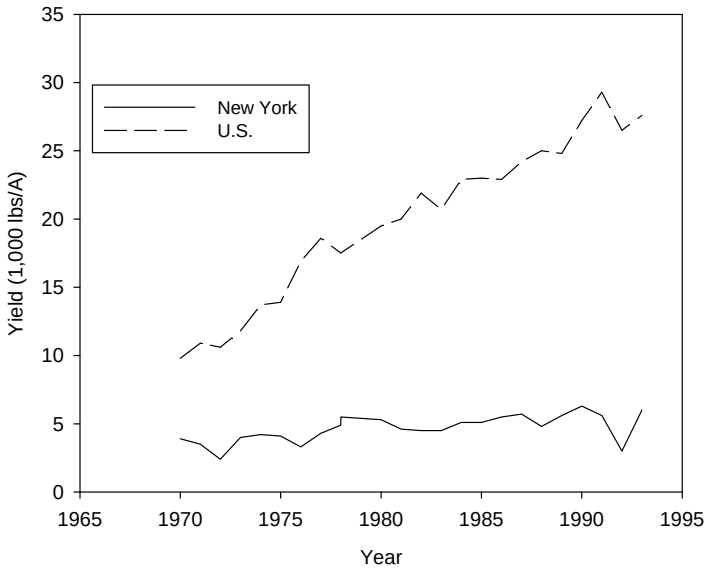

Figure 4-10 Average strawberry yields from 1970-1993 for New York state and U.S. Adapted from (Bertelson 1995)

Several other crops which have undergone selective breeding have shown significant increases in yield; soybean (*Glycine max)* with 0.5-0.9% per year (Luedders 1977), sorghum (*Sorghum bicolor* L.) with 1-2% per year (1950-1980) (Miller and Kebede 1984), maize 1.4% per year (1930-1980) (Duvick 1984), white clover (*Trifolium repens*) 0.6% per year (1930-1990) (Woodfield and Carandus 1994) and tomatoes with 0.4-1.5% per year, although the trend in tomatoes has decreased in recent years (Grandillo et al. 1999).

In most crops, yield improvements have been due to increased harvest index rather than increased plant biomass accumulation, and in some cases plant biomass has been reduced (Feil 1992). This would suggest that biomass of other plant components would have to decrease with increasing allocation to the harvested components. In the current study, growth analysis results indicate that there have been minimal changes to the carbon allocation patterns in the plant with the exception of a slight increase in dry matter of the crowns and roots (Figure 4-8). Previous studies have shown large variability in dry matter partitioning between cultivars grown in a matted row (Strik and Proctor 1988b) indicating that there has not been a focused effort to breed for specific

dry matter allocation. The same study also showed that the only growth characteristic that was correlated with yield was crown dry weight during the fruiting period. Crown dry weight was not correlated with yield in the current study; however, there appears to be an interaction between growth variables and time of measurement (Strik and Proctor 1988b). Growth analysis was conducted after harvest, a period when the crown is a large sink and is most rapidly growing (Olsen et al. 1985) which may have led to different results.

None of the canopy characteristics (LA, leaf number, leaf DW) showed any change over time or correlation with yield. Research on canopy characteristics and correlations with yield have had variable results; studies have found a negative correlation with yield and leaf number (Lacey 1973), positive correlation between leaf dry weight and leaf area in the fall with yield (Strik and Proctor 1988c) and a positive correlation between crown dry weight and yield (Strik and Proctor 1988a; Strik and Proctor 1988b). The inconsistency of the results could be due to different production systems used, time of growth analysis or the cultivars included in the study. Based on previous studies and the results of the current study, there has been little consistent change in the canopy architecture or carbon partitioning.

Results from the greenhouse study show that cultivars have maintained A_{CO2} rates that are intermediate to the two progenitor species (*F. chiloensis* and *F. virginiana*) which has been observed in previous studies (Hancock 1999; Hancock et al. 1989; Hancock et al. 2002; Sedat et al. 1989). Several studies have shown a lack of correlation between yield and A_{CO2} (reviewed by (Evans 1993; 1998)) and have led to the pervasive view that most crops are sink limited and that increasing A_{CO2} will not lead to increased yield. However, the relationship between carbon assimilation and dry matter production cannot be ignored. Dry weight accumulation of crops is related to the absolute amount of light intercepted by green foliage (Monteith and Moss 1977);

however, the effect that this has on yield is then complicated by factors such as partitioning, interleaf shading, pest and disease pressure. Long (2006) suggests that as photosynthesis is influenced by morphological characteristics which may differ between the cultivars being evaluated, the potential influence that A_{CO2} has on yield may be masked by differing plant characteristics particularly leaf area and canopy density. Further evidence of a correlation between photosynthesis and yield is that in some cases, such as maize, the increases in yield have been realized through increased biomass accumulation (Tollenaar 1991) which is directly affected by carbon accumulation (Richards 2000).

The LAR did show a significant decrease over time (Figure 4-9) due to the increased crown and root dry weight. Buttery and Buzzell (1972) suggested that a low LAR would have a larger sink for the amount of photosynthate produced and therefore, plants with lower LAR would have higher photosynthetic rates due to increased sink capacity. If the plants were sink limited, then it would be expected that the photosynthetic rate of the plants would increase with the decreasing LAR; however, in the current study, there was either no change or a decrease in photosynthetic variables (Figure 4-1, Figure 4-2).

There was no significant change in A_{CO2} or g_s over time; however, data from all three growth stages in the field trial show a decreasing trend over time with a significant response of g_s during the peak fruiting stage (Figure 4-2A, B). Due to the higher light levels in the field, A_{CO2} and g_s were higher compared to the greenhouse and values of Fv'/Fm', a measure of the effective efficiency of PSII in the light, and higher ΦPSII, were lower in the field trial. The high light levels in the field (~1500 μmol m^{-2} s^{-1}) would lead to higher photosynthetic rates, but would also result in a reduction in the efficiency of PSII due to increased amount of light energy being partitioned to non-photochemical quenching.

While the gas exchange measurements are affected by several factors that may differ between cultivars, the use of chlorophyll fluorescence can better illuminate differences in the efficiency of photosystem II (PSII). A decrease in Fv'/Fm' indicates that an increased proportion of absorbed light energy is dissipated through non-photochemical processes such as heat dissipation. Pigment molecules, particularly zeaxanthin are important in this photoprotective mechanism of heat dissipation. The 5% decrease in Fv'/Fm' observed in the greenhouse trial was not accompanied by a change in xanthophyll content which participates in heat dissipation in the leaf (Horton and Ruban 2005) indicating that the decreased efficiency was not enough to elicit increased xanthophyll production. There was a significant increase (31%) in lutein observed in the greenhouse trial. While leutin is involved in heat dissipation (Matsubara et al. 2005), the contribution is minimal and did not likely contribute to changes in fluorescence.

In the greenhouse trial, the progenitor species *F. chiloensis* had significantly higher rates of photosynthesis, Fv'/Fm' (Figure 4-1), higher amounts of chlorophyll and lower xanthophyll content (Table 4-2). *F. virginiana* had rates that were lower than cultivars and the trend of decreasing Fv'/Fm' and ΦPSII observed in the greenhouse trial seem to be bringing the values closer to that of *F. virginiana.* As *F. virginiana* is native to the northeastern U.S., characteristics that would make a cultivar successful in this region would likely be found in *F. virginiana.* Characteristics related to photosynthetic capacity may be unintentionally selected for when selecting for other traits such as disease resistance.

ΦPSII showed a decreasing trend in both the greenhouse and the field trial. ΦPSII is affected by processes that compete for electrons; N metabolism, photorespiration and the Mehler reaction (Maxwell and Johnson 2000). It is therefore a sensitive measure of the efficiency of the photosystem.

Another major sink for electrons is photorespiration, which is influenced by leaf age, water stress, temperature, fruit load and genotype (Perry et al. 1983; Salin and Homann 1971). Water status and temperature would have been the same for all plants in the current study and there was no correlation between fruit load and ΦPSII. Genetic differences in the amount of photorespiration could have resulted in different ΦPSII values. An increase in metabolic processes that compete with photochemical quenching could result in a reduction in the amount of photosynthate produced. Low photosynthate supply has been shown to reduce the accumulation of sugars in the endosperm of maize (Setter and Flannigan 1989) and may have contributed to the reduction observed in soluble solids in the fruit.

Although the overall yield of cultivars appears to have undergone limited improvement, there have been significant changes in fruit size during the last century. The average fruit size of both the king and lower order berries have steadily increased (Figure 4-4). The gains in fruit size have not been realized in yield due to the concomitant reduction in berry number (Figure 4-5). The reduction in berry number is consistent with lower flower number observed in more recent cultivars (Figure 4-7); however, flower number has decreased much more (-50%) compared to the reduction in berry number (-18%) indicating that fruit set has significantly improved over time. Increases in fruit size can contribute to increased yield; however, significant increases in yield potential will likely require increased fruit number as well (Lacey 1973). This has been observed in tomato (Grandillo et al. 1999) and several grain crops (Feil 1992) where increase in yield has been due to increased fruit number rather than size.

Not surprisingly, the most pronounced change in the fruit was observed in a characteristic that has been intentionally bred for, fruit firmness. An increase of 98% has occurred over time (Figure 4-6). There was also a significant decrease in the percent soluble solids over time (Figure 4-6). Previous studies have found that cultivars

vary greatly in the amount of soluble solids which is also affected by ripeness, fertilization, irrigation and genotype (Kays 1991). Heritability studies on California strawberry cultivars have found that the selection response for soluble solids is highly affected by the environment the selection occurs in but it is possible to select for improved content, whereas selection for low soluble solids did not have a significant response (Shaw 1990). This would suggest that the decreasing trend observed in this study is likely a secondary effect resulting from selection for a different trait.

The health components of fruit are becoming increasingly important to consumers, but have not been a characteristic that has been bred for in the cultivated strawberry. There was large variation observed between cultivars, with an almost three-fold difference in anthocyanin content and almost two-fold difference observed in the phenolic content of the fruit (Table 4-3). There was no significant correlation between content and size of fruit, so the differences observed are not likely due to a dilution effect. Previous studies indicate that cultivar can have a large influence on the content of phenolics and anthocyanins (Heinonen et al. 1998; Maas et al. 1991). These results suggest that breeding has not resulted in a change in total content; however, there is evidence that the composition of the specific compounds in cultivars are significantly different from the progenitor species (Aharoni et al. 2004). Additional analysis of individual compounds in the cultivars may elucidate changes in composition that have occurred over time.

Summary

There has been little improvement made in northeastern strawberry cultivars during the last 30-40 years except for firmness and disease resistance. This is demonstrated by the continued prevalence of cultivars such as ‘Jewel’ (1985), ‘Honeoye’ (1979) and ‘Allstar’ (1981) in the market today. There are several factors

that may be contributing to this apparent ceiling in productivity and quality, and several avenues, addressing both short term and long term goals should be explored.

One possible avenue to improving yield is to put a greater focus on canopy architecture and carbon partitioning during the breeding process. Optimal canopy architecture for light interception and carbon partitioning has been correlated with significant yield improvements in several other crops (Duncan et al. 1978; Duvick and Cassman 1999; Feil 1992; Irvine 1975). A dense plant canopy can result in greater disease pressure as well as greater interleaf shading within the canopy which increases the number of leaves that become sinks. This demand for photosynthates could be significant if the plant is indeed source limited, which is suggested by the lack of response to increasing LAR observed.

Increasing the number of fruit produced should also be explored as increases in yield are often due to increased fruit number rather than size. There is a great deal of genetic variability in fruit number available in both older breeding stock as well as wild *F. virginiana* species that could be utilized to achieve this objective.

There also are more long term goals that could greatly enhance the yield potential of the strawberry. The first is the incorporation of new germplasm into the breeding stock. Several studies (Dale and Sjulin 1990; Hancock et al. 2002; Sjulin and Dale 1987) have demonstrated that the genetic variability in the cultivated strawberry is narrow. This narrow germpool may be contributing to the lack of improvement that has been achieved through breeding with the current breeding stock. Introduction of wild species with desirable characteristics such as: carbon partitioning, fruit characteristic and flower number that should be occurring in any breeding program.

Another long term strategy would be to further explore the role of photosynthesis in limiting productivity. This study has shown that there has been no

change in photosynthesis in cultivars released during the last century. The cultivated strawberry has photosynthetic rates that are intermediate to the progenitor species, *F. chiloensis* and *F. virginiana*, and breeding studies have shown that the high photosynthetic characteristics of *F. chiloensis* may be a quantitatively inherited trait (Hancock et al. 1989) and may therefore represent a possible source for improving the photosynthetic capacity of the cultivated strawberry.

The need for disease resistance has certainly contributed to the comparatively low improvement in productivity; however, there is clearly a need and potential to increase the productivity of the northeastern cultivars. Through the manipulation of carbon allocation in the current germplasm as well as the introduction of new genetic material, the apparent productivity plateau can be broken.

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CHAPTER 5

COMMUNICATION AND RELATIONS BETWEEN SMALL FRUIT GROWERS AND RESEARCHERS IN NEW YORK STATE

Introduction

New York state berry growers represent a small portion of the agricultural industry in New York State which is dominated by dairy, greenhouse, hay, cattle and apple production (USDA, 2005). There are over 2,400 acres in strawberry and blueberry production in New York State with a market value of over $10.3 million; however, recent studies which have shown the great health benefits of berries have led to increased demand and production in the future. The agricultural research community has played a key role in developing systems for more efficient production. For example, mechanization of harvesting in the blueberry, cranberry and raspberry industry has drastically reduced labor costs. In New York State, many of these new techniques are introduced to growers through the cooperative extension programs. Historically, the information generated through the research is disseminated in bulletins and by the extension educators via farm visits and workshops. With increased access to the internet, many of these resources as well as resources from other agencies are available on the internet.

The original conception of the Agricultural Extension Service was that the extension agents would act as the liaisons between the growers and the academic community: "Extension field staff bring the needs of various stakeholders to the attention of Land Grant university researchers, who then pick which problems they want to address, investigate them, and provide research results to the extension educators to take back to stakeholders. Essentially, research drove the research-

extension model, though, in theory, researchers were receptive to input from the field." (Decker 2004). This model of information transfer with a top-down approach has been the dominant model and is "based on the assumption that new agricultural technologies and knowledge are typically developed and validated by research scientists, and that the task of extension agencies is to promote the adoption of these technologies by farmers, thereby increasing agricultural productivity." (Black 2000). This structure situates the researchers as the sole proprietor of new innovations and the farmer as the recipient rather than participant in development.

Russell (1989) concluded that: "Farmers have almost universally been sold short as both competent scientific thinkers and researchers. The upshot of this tendency has been to perpetuate the modern myth that research is the exclusive domain of professional researchers and that farmers are inevitably on the receiving end of their work….Farmers have a strong desire to participate in setting the research agenda and are increasingly willing to be co-researchers with the scientists. The strength of any joint management of research projects is that R&D can become an integrated activity. The adoption of research findings is then a critical issue from day one of the investigation rather than the final and problematic step of agricultural research."

The berry industry in New York State has a very active grower organization, the New York Berry Growers Association, which has worked with berry researchers in developing research priorities and also funding some research projects. Researchers also interact with growers through field days, workshops and conferences; however, these interactions typically involve the researcher relaying research results or instruction and there is usually not enough time for feedback from growers on research objectives. This structure of interaction between researchers and growers has allowed for growers to have some influence over the research priorities that drive the research

programs, but as the industry is changing, it is difficult to determine which representatives of the industry are involved.

The objectives of this study are to define the demographics of the berry growers in New York State, to determine what their participation is in the research process and to determine the sources and channels of information that growers utilize. By understanding the grower's perspective of their role in setting research priorities and how they access information, programs and strategies can be developed that encourage participation of all growers in the research process so that all growers in the berry industry have equal participation in the research process if they choose.

Materials and Methods

Target Groups

1) New York State Berry Growers Association

The NYBGA is composed of 130 berry growers across New York State which procures funds through membership dues, sales of row cover and contributions from growers in order to provide services for the growers. Funds are allocated to marketing, publication of newsletters, administrative costs and research. The funds allocated to research in 2005 totaled $4,500 (28.3% of income). In order to determine the projects that funding is allocated to, the association asks their members to prioritize what they view as the most important aspects of research for the coming year. The NYBGA holds annual meetings at which the results of the research projects they have funded are reported back to the association.

2) Northeast Organic Farming Association of New York (NOFA-NY) Berry Growers

NOFA-NY is the primary organic farm certification program in New York State and has 70 certified growers that grow berry crops. Several of the growers in this group do not grow berry crops exclusively but also produce mixed vegetables, other types of fruit, herbs, spices, and various other crops. The NOFA-NY works with CCE whom is involved in certification information sessions as well collaborating on research projects with Cornell University.

Data Collection

. There were two main forms of data collection used; a survey and interviews. The survey was delivered to selected recipients by both mail and email. Participants could fill in the paper copy and mail it back in the prepaid envelope, or they could complete the survey online. All participants were given both options. Reminder postcards were mailed and emailed to all recipients who had received the survey but had not yet responded two weeks after the initial mailing. All mailed in surveys were entered into the online survey program. The survey was used to collect information about demographics, production techniques, organizational involvement, resource utilization and research involvement.

Interviews were conducted with survey participants that indicated that they would be willing to be contacted about participating in the interview. The interviews allowed for a more candid investigation of the views of the different groups involved in small fruit production. The objective of the interview was to spend time with the growers in order to develop an understanding of the factors that have influenced the direction and decisions they have made and how those decisions have impacted their business plan as well as their relationships with the community, other growers and academia.

Results

Survey Data The experience of the growers that responded to the survey was evenly distributed from less than 5 years to more than 20 years (Figure 6-1A). The majority of growers (40%) use a low spray or judicious spray program, over 30% of growers are organic or no spray program.

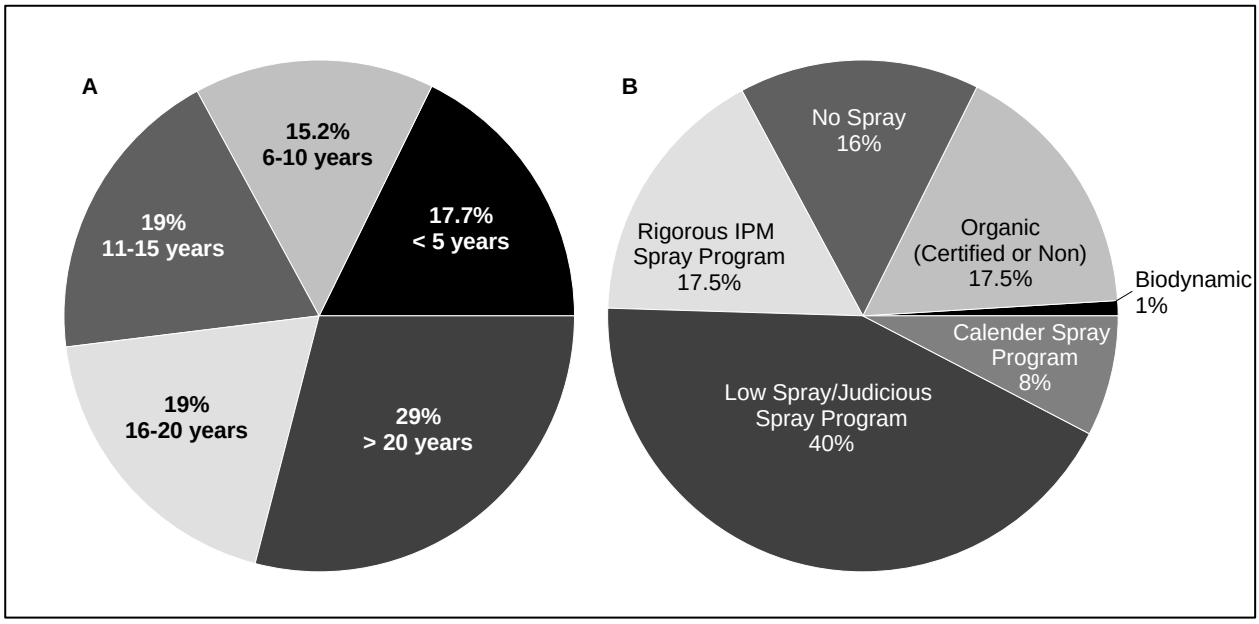

Figure 5-1 Years of experience of respondents (%) (A) and production system used by growers (% respondents) (B)

The majority of growers (44%) had 20 acres or more in total production, however for berry production, most growers (~56%) had 3 acres or less (Table 6-1.)

Table 5-1 Total acres and acres in berry production of respondents.

Acres	TotalAcres (% respondents)	Berry Acres (% respondents)
< 1	7.7	25.6
1-3	19.2	30.8
4-6	7.7	15.4
7-10	6.4	9.0
11-20	14.1	9.0
> 20	44.9	10.3

The primary markets used by most berry growers are pick-your-own (PYO), farm stand and farmer's markets (Figure 0-2). Almost 100% of the respondents rely on word of mouth. Signs and newspapers are also used to a large extent. Less than 30% used websites and 20% of respondents use email as an advertising tool (Figure 6-2B)

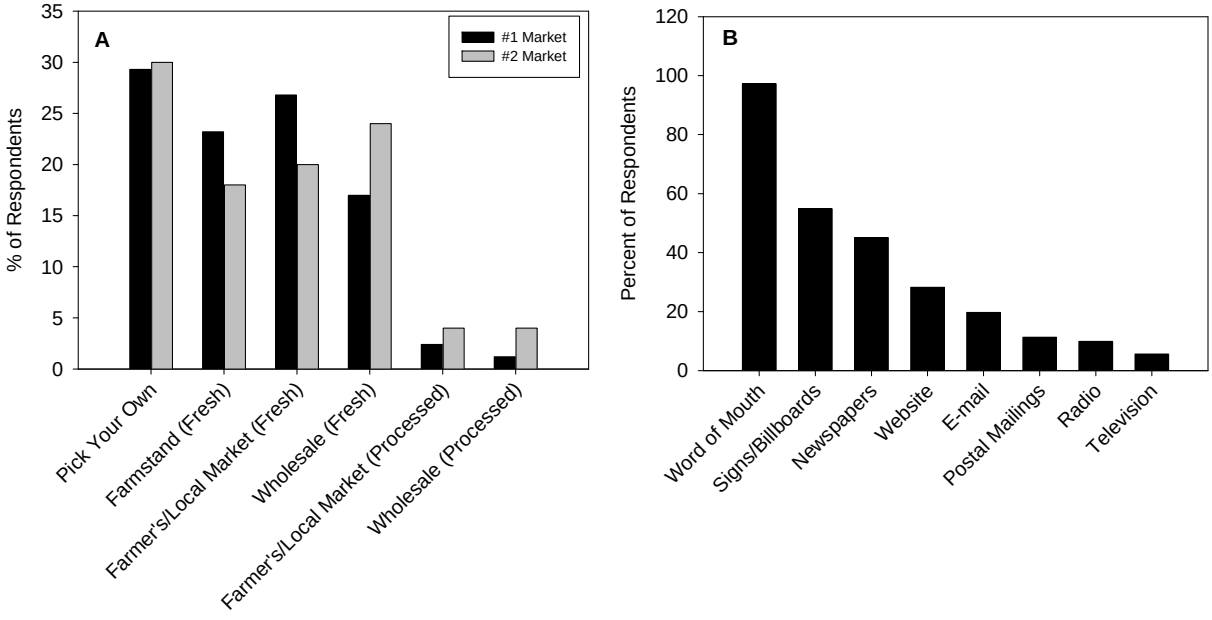

Figure 5-2 Primary and secondary markets of growers (A) and forms of advertising used by growers(B).

When growers were asked to rate the importance of information sources, personal experience, extension educators, fellow growers and production guides were ranked as the most important. However, there were some differences between the small growers (<3 acres) and the large growers (>10 acres), 56% of small growers rated extension educators as very important compared to 46% of large growers. Other resources typically provided by the extension also had different ratings. The percentage of small growers that rated workshops and field days as very important were 27% and 17%, respectively compared to 40% and 27% of large growers. Private consultants and the Northeast Organic Farming Association (NOFA) resources were not considered important by most growers (Table 6-2). The credibility of these sources was ranked similarly to their importance (Table 6-3).

Table 5-2 Importance of information sources to berry growers.

	Very Important	Somewhat	Minor	Not Important
Personal Experience	86.8	11.8	1.5	0
Extension Educators	66.7	31.9	0	1.4
Fellow Growers	45.3	32.8	18.8	3.1
Production Guides	56.9	38.5	3.1	1.5
Hardcover Publications	31.3	42.2	21.9	4.7
Newsletters	47	43.9	6.1	3
NOFA	5.8	17.3	40.4	36.5
Cornell Fruit Resources Website	37.3	44.1	5.1	13.6
Other Internet Sources	19.7	34.4	21.3	24.6
Workshops/Conferences	36.9	43.1	13.8	6.2
Field Days	26.2	44.3	18	11.5
Private Consultants	16.4	27.3	18.2	38.2
Trade Magazines	13.6	42.2	28.8	15.3

The largest difference in information access between the large and small growers was the extent to which they used internet sources. 19% of small growers rated the Cornell Fruit website as very important compared to 27% of larger growers, but 38% of small growers also rated other internet websites as very important compared to 0% of large growers.

Table 5-3 Credibility of information sources.

	Very Credible	Somewhat	Minor	Not Credible	N/A
Extension Educators	64.3	24.3	4.3	3	7.1
Fellow Growers	34.3	41.8	13.4	0	7.5
Production Guides	62.1	30.3	3	1.5	4.5
Hardcover Publications	33.3	31.8	12.1	0	21.2
Newsletters	41.5	44.6	4.6	3.3	9.2
NOFA	3.3	20	16.7	1.5	56.7
Cornell Fruit Resources Website	47.7	20	3.1	4.8	27.7
Other Internet Sources	12.7	36.5	11.1	3.2	34.9
Workshops/Conferences	37.1	40.3	8.1	1.6	11.3
Field Days	29	37.1	6.5	1.6	25.8
Private Consultants	21.3	18	6.6	1.6	52.5
Trade Magazines	9.4	40.6	26.6	15.3	21.9

Overall, 35% of growers indicated that they strongly or slightly agreed that they were aware of the research objectives and projects currently ongoing in the state. When large and small growers were compared, only 15% of small growers strongly or slightly agreed that they were aware of research objectives compared to 54% of large growers.

48% of large growers slightly or strongly agreed when asked if they were satisfied with the input they had in setting research priorities, whereas only 28% of small growers were satisfied with their input. For questions pertaining to satisfaction with their lifestyle, size of operation was not a factor and response was primarily positive. When asked about their satisfaction with their lifestyle, if they were optimistic about the future and their community's appreciation of what they do the responses were primarily positive

.

Table 5-4 Percent of respondents indicated for questions regarding research awareness, involvement, community appreciation and attitude towards the future of berry farming in New York State.

	Strongly Agree	Slightly Agree	Neutral	Slightly Disagree	Strongly Disagree	Not Sure
I am aware of the research objectives and projects relating to berry production occurring at reserach institutions in NYS	11.6	24.6	29	5.8	14.5	14.5
My primary concerns relating to berry production are currently being addressed by research institutions in NYS	7.4	25	23.5	2.9	0.0	41.2
I am satisfied with the input that I, as a grower, have in decisions regarding research priorities in the area of berry crops.	9.1	33.4	22.7	6.1	4.5	24.2
I have collaborated with a berry crops researcher/extension educator at least once during the past year.	29.3	16.9	9.2	3.1	27.7	13.8
I am satisfied with my current lifestyle (socioeconomic position) as a grower.	26.2	26.2	16.8	15.4	10.8	4.6
I feel that my community appreciates my contributions as a grower.	41.2	30.9	16.2	2.9	4.4	4.4
I am optimistic about the future of berry farming in NYS	38.2	33.8	13.2	5.9	1.5	7.4

Interview Data

The interviews were used to supplement the information gathered through the surveys and to better understand the dynamic between growers and researchers. There were no organic growers that agreed to participate in the interview process, so the interviews represent non-organic growers. However, although not certified organic, 4 of the 7 growers interviewed indicated that they do produce some of their crops organically. The remainder all used a rigorous IPM program. Half of the growers interviewed came into farming after a non-agricultural career while the other half had grown up in an agricultural community.

All of the growers interviewed did not start with the intention of growing berries, but started with an orchard or a dairy farm and decided to diversify their crops by adding a high value berry crop. As identified in the survey data, most growers have diversified operations including fruit trees, flowers, or vegetables. Two growers are growing exclusively berries. Two of the growers also had off-farm income that supplemented their income. The primary market, as identified in the survey is direct market through a farm stand or farmers market, although one grower is considering wholesaling strawberries in the future.

The sources of information the growers accessed were similar to the survey data in that the top source was CCE and very little use was made of official organic sources. However, the interviews also indicated that the use of CCE was strongly dependent on the connections the grower had to organizations such as Cornell Cooperative Extension (CCE) and the New York Berry Growers Association (NYBGA). Four of the growers had either worked for CCE or participated in Master Gardener School and all of these growers indicated that the CCE was their primary source of information. These growers also indicated that the information given by CCE is very credible. For example, the following quote were taken from interview transcripts:

"If it comes from the University, I take it the way it is."

"All my information comes from CCE, no doubt"

"When you get information from the extension service, you know you are getting ` the right stuff."

If growers were not somehow connected to the organizations mentioned, there was little use of CCE resources and these growers tended to utilize trade magazines and industry members. Even though the survey indicated that trade magazines were ranked second lowest for importance and the lowest for credibility, interviews with the growers revealed that all the growers interviewed utilized trade magazines as an important source of information. Only two of the growers mentioned that other growers were a valuable resource they communicated with on a regular basis. The only interaction growers had with each other occurred at farm visits organized by the CCE or at the annual New York Fruit and Vegetable Expo.

Growers were asked about how much input they felt they had in setting research priorities and expressing concerns to those involved in research. Those growers that were very involved in the NYBGA, which were also the growers that had larger operations and grew exclusively berries, felt that they had a lot of input into the setting of priorities and were very satisfied with the role they played. The growers that had smaller plots of berries and grew a wide variety of crops did not feel that they had a lot of input in research; however, they also felt that perhaps they should not have that much input in research as they had only a small acreage of berries. Growers in remote locations indicated that they did not have much interaction with research as they were located so far from the extension office and did not interact with extension educators.

Discussion

The objective of a land grant university research program is to meet the needs of the stake holders they serve. Extension research priorities should be based on the

premise that the stakeholders in agricultural research and extension are involved in setting priorities and developing research agendas (Farrington 1998). One of the challenges in doing this with the berry industry in New York is that the growers are not a homogenous group. As the survey results indicate, most of the growers have three acres or less in berry production and have diverse operations that may include; tree fruits, flowers, vegetables and herbs. The New York Berry Growers Association is the organization that is most vocal in communicating priorities to the researchers. The members of this organization also have some additional influence on the research conducted through their research funding program. This makes it much easier for researchers to get input from growers as there is an organized point of contact. Farrington (1998) has observed that when farmers are part of a larger organization and have more specialized production, the input into research priorities are farmer driven and tend to have more influence as they are an organized voice. It is easier for extension to meet the demands of these groups as they tend to be more focused and specialized in a specific commodity. However, the results of this study show that most growers are no longer in this category and therefore, the traditional reliance on grower-driven participation in research and communication may no longer be adequate to meet the needs of the entire community.

There are many forms of communication that have been developed in extension over the years, with over 65 channels of communication identified (Waller et al. 1998). However, it has only been the last 20 years that the importance of including the farmer in the process of research and development has been recognized (Rhoades and Booth 1982; Whyte 1991). In order to effectively include growers in the research process, it is necessary to identify which forms of communication are effective for all members of the stakeholder group.

Information access by farmers has been extensively studied over the last 80 years (Boone et al. 2000) and several theories have been developed to understand what the most effective forms of communication are. One of the original theories of how information is disseminated is the diffusion theory, which is based on the premise that innovations create both opportunity and uncertainty and the role that information plays is to reduce the uncertainly (Rogers 1995). A useful aspect of this theory is that there is a clear distinction drawn between the *source* of information, which provides the content and expertise of interest to a specific sector and the *channel* of information which is the vehicle through which the information is conveyed (Tucker and Napier 2002).

The channel used can have an influence on how the information is perceived, as is demonstrated in a study using the 'Risk Communication Theory' which showed that the perceived risk of chemical use was influenced by the source of the information. Other studies have shown that individuals that access information from popular media such as television, newspapers and neighbors have a greater perception of risk in regards to health and household hazards (Coleman 1993; Loges 1994). In a similar study evaluating agrochemical risks, it was found that farmers had lower perceived risk of agrochemical use if the information came from the Soil Conservation Service rather than other sources (i.e., chemical dealers, other farmers, extension service, EPA, etc). The different impact of the information sources may have been due to the nature of the SCS service and the relationship it had with farmers. The SCS is a service that also provided local, farm-specific advising. As a result, they understood the local constraints and conditions, their literature offered technical, practical ways to address issues, therefore, SCS is perceived as an objective, accessible source to farmers. In addition, farmers have to work with the SCS due to their role in policy development (Tucker and Napier 1998). This is an example of the importance of how the information is presented (i.e. with a solution vs. simply warning about a potential

problem) as well as the relationship that the grower has with the organization (farmers had to work with SCS to meet policy requirements). Not only identifying these relationships, but understanding how the dynamic affects communication, is an important aspect if trying to improve grower involvement in research.

New York berry growers can be categorized into two main groups; larger operations with 10 acres or more that are specialized in growing berry crops and those that are smaller, with 3 acres or less in berry production and typically have more diverse operations. Survey results indicate that the latter group is the largest segment composing 55% of the berry industry in New York State. This is likely due to the increased number of growers that are currently producing other commodities such as tree fruits and vegetables that are diversifying their production systems by adding berry crops. This is also reflected in the distribution of years of experience. There is an even distribution from less than 5 years of experience to over 20; this is expected as the berry industry has been steadily growing in recent years. The type of production system utilized is also quite diverse; however, most growers identify with a low/judicious spray program. Of the growers surveyed, 17% identified with an organic production system all of whom were small operations. Several of the growers that were interviewed indicated that they grow a portion of their berry crops organically, even though they do not identify as an organic grower, so the portion of growers that utilize organic practices in some aspect of their production system is likely higher than reported.

Categorization according to size also illustrated differences in membership in the New York Berry Growers Association. 66% of large growers were members compared to 33% of small growers. The NYBGA has been a valuable organization for both growers and researchers. However, most of the smaller growers, which now compose the majority of berry growers in New York, do not participate in this organization and there is an indication that this demographic is growing. In the case of

smaller growers, who often have a more diverse production system, there is less interaction and therefore less input into the research process. This was observed in the interviews, where growers who only had a few acres of berries felt as though their input was perhaps not as valuable as they only had a few acres in berry production and in some cases did not know what venue was available to communicate their concerns.

> "I'm not a huge grower, so I don't know how much input I really should have."
>
> "I have no input at all, I think I am just too small, most of the research is geared towards the larger operations."
>
> "I'm not sure who I would talk to if I wanted to share my thoughts on what research should be done."

In the case of growers with diverse, smaller operations, involvement in the research process is not likely to be grower driven. As they often have more types of crops growing, there is less time to devote to a specific crop and in many cases, a small grower will also have an off-farm income which demands time. Therefore, getting input into the research process from these more marginalized growers may require greater initiative on the researcher's part, which has been observed in previous work on low-income farmers (Farrington 1998).

As was indicated by the interviewees, the survey data indicated that 54% of large growers were aware of the research objectives of berry programs and 46% were satisfied with the input they had in setting research priorities. In contrast, only 15% of small growers were aware of research objectives and 28% were satisfied with their input in setting research priorities. The relationship observed in this study between size of operation, involvement in grower associations and subsequent involvement in setting research priorities is consistent with previous work (Farrington 1998; Waller et al. 1998) and is an important relationship to consider when developing an extension

program and determining the most effective forms of communication to ensure adequate representation of all stakeholders in the state.

These differences observed between large and small berry farms are consistent with the framework of the farm structure theory which uses characteristics of the farmer and farm operation (i.e., age, size of operation, education and debt-to-asset ratio), of which size of operation is the best predictor (Waller et al. 1998), to determine information utilization (Napit et al. 1988; Nowak 1992; Waller et al. 1998). There were a few key differences in the information sources accessed by growers of different sized operations. Although fewer (-10%) of the large growers rated extension educators as very important, the number of large growers that rated workshops and field days, which are typically organized by CCE, as very important were 13% and 10% higher compared to the smaller growers.

Methods of interpersonal communication, such as workshops, field days, and extension agents were overall ranked as important and credible sources of information. The use of interpersonal, direct contact formats was the basis on which extension programs were first developed and have continued to be an important aspect. Farm tours, shows and demonstration plots have been shown to be not only popular, but also be very effective in getting growers to adopt new technologies (Johnson 2000). The greatest challenge with interpersonal communication is the time and resources required, particularly when the clientele are located over a large area. The increasing accessibility of the internet, the 24 hour access to the information and the ability for two-way communication may replace or supplement current interpersonal forms of communication (Tucker and Napier 2002). The use of the internet holds great potential to improve the dialogue between growers and researchers and allow for the involvement of growers that are currently not engaged in the communication with researchers. In the current study, one of the most striking differences between large and small growers was

observed in the usage of web-based resources. A greater percentage of the larger growers (27%) ranked the Cornell Fruit resources webpage as very important compared to 19% of the smaller growers; however, none of the large growers rated other internet sources as important compared to 38% of small growers that indicated this was a very important resource. This is likely due to the stronger connection that large growers have with the research and extension community through their involvement in grower organizations. As they are involved in the research process, they are more likely to trust and also know about the information that is available on the Cornell website as opposed to other online sources.

Farm magazines have been shown to be a preferred channel of information in previous studies (Hartke 2001; Richardson and Mustian 1994). In the current study, only 14% of survey respondents ranked magazines as a very important resource and 42% ranked them as somewhat important and credibility ratings were similar. This lower ranking of magazines compared to other studies may be due to the smaller number of publications that are specific to berry crops compared to some of the more major crops.

The Way Forward

The results of this study have shown that the berry growers in New York State are a diverse group that differs not only in the type and size of operation, but also in their utilization of resources and participation in research. The current method of acquiring input in the research process from growers, which has been largely through surveys administered through the New York Berry Growers Association, may be resulting in an over-representation of growers with larger operations. As illustrated in the current study, the smaller growers, which represent 55% of the industry, are not involved in grower organizations and as a result, do not have as much connection with

the research process. The results also indicate that the sources and channels of information that the smaller growers access differ from the larger growers.

Based on the results of this study, there are avenues that can lead to increasing the representation of small growers in the research process. While it is essential to continue service to the large growers in the community and maintain the interaction with NYSBGA as they represent a large component of production in the state, there must also be an increased effort to engage the small growers. In order to accomplish this, researchers and extension agents must reduce the reliance on grower-driven participation and put more effort into engaging the smaller growers who do not and are not likely to participate in the associations.

Engaging the smaller growers may require greater initiative on the research and extension community. The diversity of crops and the limitations on the grower's time will necessitate a venue for communication that will allow them to gain information about research as well as a simple portal to allow these growers to offer feedback to researchers. A logical channel to meet these needs would be the internet. According to this study, small growers access web-based resources to a much greater extent compared to the large growers. The internet has become an increasingly important tool in extension and has been extensively utilized to disseminate information to growers. One of the challenges with using internet as a communication tool is that it is typically used as a one-way communication. However, there are increasing numbers of applications that can be incorporated into a website that enable discussion and feedback forums, such as bulletin boards where users can post comments or discussion forums which could be used to have scheduled on-line forums when researchers and extension agents will be available online to interact with users.

Currently, Cornell has two websites that would be logical hosts for such communication tools: the Cornell Fruit Resources website and the Cornell Small Farms website. This approach to interacting with small farmers across the state addresses some of the challenges with extension work; it allows the small farmers access to a venue at any time and without having to travel to a workshop or field day. Likewise, the researchers and extension agents can reach a vast number of growers they would not otherwise be able to interact with due to resource and time constraints.

The increasing interest in producing berry crops is an encouraging sign for the berry industry and the increasing diversity of the growers presents research and extension efforts with the challenge of meeting a diversity of needs. The results of this study give a good indication of ways in which the research community can evolve to meet the needs of all the stakeholders in the state and do so in an equitable and collaborative way.

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