Carbon and Nitrogen Reserves in Perennial Strawberry Affect Plant Growth and Yield

Laura Elisa Acuña-Maldonado and Marvin P. Pritts¹

Department of Horticulture, Cornell University, Plant Science Building, Ithaca, NY 14853

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ABSTRACT. Early spring growth of perennial strawberry (*Fragaria ×ananassa* Duch.) plants is supported by the carbohydrate and nitrogen (N) reserves accumulated from the previous growing season. The limitations of these reserves on the initial spring growth and yield of perennial strawberries have not been studied in detail, particularly the influence of N reserves. Differential N fertigation (0 to 20 mM N) was applied to potted strawberries during the growing season and a supplemental foliar urea application was applied to a portion of the plants in the fall to modify reserve N during dormancy. Plant N content and spring vegetative growth the year after fertigation increased nearly twofold with increasing N fertigation. Photosynthesis per unit leaf area also increased up to 10 mM of fertilizer N and then stabilized through 20 mM. Foliar urea application in fall further increased total plant N content and size, decreased carbohydrate concentration, and also decreased yield in plants with the most total N. Nitrogen fertigation was resumed on a portion of these plants in early spring, but new growth and subsequent yield were unaffected by spring N application. In a second experiment, CO₂ enrichment with and without soil and foliar N application in the fall was used to vary carbon (C) and N reserves. CO₂ enrichment in fall increased plant size and yield the next July by ≈20%, but total nonstructural carbohydrate and N concentrations were unaffected. Foliar urea application also increased N and C reserves (but not concentration) as well as yield in both enriched and unenriched plants. Although foliar urea in fall decreased carbohydrate concentration, total reserve levels were unaffected because treated plants were larger. In this experiment, spring N increased plant size by ≈50%, but yield was increased only 12%, suggesting that yields are mostly dependent on reserves. Increasing N reserves with a late fall foliar application is one strategy growers can use to efficiently enhance growth and yield in low to moderately fertilized plants.

At the end of the growing season before the onset of dormancy, many deciduous plants, including perennial strawberries, accumulate carbohydrates and nitrogen (N) and use these reserves to support initial growth in the spring (Bushway and Pritts, 2001; Cheng and Fuchigami, 2002; Titus and Kang, 1982; Tromp, 1983). Nonstructural carbohydrates constitute the bulk of the total carbon (C) reserves, whereas N in the form of proteins and amino acids usually represents less than 4% of the dry matter in a strawberry plant (Marschner, 1986; Mengel and Kirkby, 1987). As spring growth begins, both reserve carbohydrate (Bushway and Pritts, 2001; Priestley, 1960; Worley, 1979a) and N concentrations (Taylor, 1967; Tromp, 1983) decrease.

Early fall defoliation has been used to experimentally manipulate reserve carbohydrates to determine their effects on spring regrowth (Hennerty and Forshey, 1971; Loescher et al., 1990; Sparks, 1977; Worley, 1979b). However, Cheng and Fuchigami (2002) found that early defoliation also prevents N remobilization from leaves to storage organs and reduces root N uptake, which is predominantly supplied by mass flow and depends in part on the volume of water transpired. Thus, early fall defoliation may induce N limitations in the subsequent spring as well. To understand the direct effects of C and N reserves on growth and yield, these variables have to be manipulated independently.

Manipulation of N supply through fertigation has been achieved successfully in crops such as apple (*Malus ×domestica* Borkh.) (Cheng and Fuchigami, 2000) and grapes (*Vitis labrusca* L.) (Cheng et al., 2004; Xia and Cheng, 2004) using potted plants and a coarse-textured soil medium. Researchers also have used low concentrations (3% to 10%) of foliar urea solutions applied in the fall to increase N reserves (Cheng and Fuchigami, 2002; Khemira et al., 1999; O’Kennedy et al., 1975; Oland, 1963).

Plant N status appears to influence the capacity to respond to additional fertilizer N. For example, more N derived from the foliar urea is partitioned into the root system of low N trees than high N trees (Cheng et al., 2002). Yield responses to foliar urea in strawberries have been reported under very low nutrient conditions (Albregts and Howard, 1980, 1986). However, in field studies by Breen and Martin (1981) and Strik et al. (2004), little response to N fertilizer was found in strawberry. Although the ability of N to influence growth and yield of plants with low N status is clear, effects of N on plants with a higher N status is not completely understood, particularly because N status also can affect carbohydrate status (Cheng and Fuchigami, 2002).

Carbohydrate reserves can be either increased or decreased by increasing N availability. When Rubisco is limiting, an increase in N can lead to increased CO₂ assimilation because more Rubisco will be manufactured. In contrast, if Rubisco is not limiting, further increases in N availability can decrease carbohydrate status because decarboxylation of carbohydrates is required for N assimilation (Burkhardt, 1938; Cheng and Fuchigami, 2002). Carbohydrate status also can be increased directly through exposure of plants to elevated levels of CO₂. Exposure to high CO₂ stimulates net C assimilation and leads to an increase in carbohydrate levels in leaves (Bushway and Pritts, 2001; Chen et al., 1997a). Yields might be enhanced if

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¹Corresponding author. E-mail: mpp3@cornell.edu.

increases in CO₂ assimilation rates could be sustained during critical phenological stages, for example, during strawberry flower induction (September) or at the time of fruit filling (June to July).

We hypothesized that if N is limiting, then increasing N reserves in fall will increase yield the next spring. Also, if C is limiting, then increasing C reserves in the fall will improve vegetative growth and fruiting the next spring. These two hypotheses are somewhat related. In circumstances in which N is adequate to sustain the photosynthetic machinery necessary to meet plant demand for carbohydrate, additional N could depress carbohydrate levels (and subsequently growth and yield) because carbohydrate is required for N assimilation.

The objectives of this work were 1) to determine if fall N and/or carbohydrate reserves limit spring growth and yield in perennial strawberries; 2) determine if spring N applications can replace reserve N; and 3) determine if an interaction exists between N and C levels.

Materials and Methods

Dormant, single-crowned, certified plants of 'Jewel' were obtained from a commercial nursery. Bare crowns were planted on 1 May 2001 and each one set in a 3.8-L plastic pot containing sterile sand (pH 6.5) and grown outdoors under full sunlight. Pots were spaced at 30 × 20 cm in a nursery at Cornell Orchards, Ithaca, NY (lat. 42°26'26" N, long. -76°29'47" W). Plants were watered as needed and assigned randomly to each of two experiments.

EXPT. 1: INCREASING NITROGEN RESERVES. Starting on 10 May 2001, all plants were fertigated weekly through irrigation with a solution (pH 6.6) containing 14 mM N using Peters® 20N-8.7P-16.6K with STEM® micronutrients (J.R. Peters, Allentown, PA) for 5 weeks until plants were established. Flower buds were removed on 21 and 28 May 2001, and during the summer, plants were derunnered weekly. Both flower buds and stolon tissue were discarded.

Beginning on 15 June 2001, plants of similar canopy size were selected and randomly tagged to receive one of five different N concentrations (0, 5, 10, 15, or 20 mM). Plants were fertigated twice weekly with the assigned rate for 43 d by applying 300 mL of a complete nutrient solution to each pot using a modified Hoagland's solution No. 2 (phosphorus, potassium, magnesium, and calcium were supplied from stock solutions containing 4 mM of KH₂PO₄, MgSO₄·7H₂O, and CaCl₂·2H₂O) (Cheng and Fuchigami, 2000; Hoagland and Arnon, 1950) so that N was supplied solely from NH₄NO₃. Micronutrients were supplied through STEM® (0.54 mg·L⁻¹ boron, 0.91 mg·L⁻¹ copper, 2.97 mg·L⁻¹ iron, 0.016 mg·L⁻¹ molybdenum, and 1.78 mg·L⁻¹ zinc). Pots were arranged in a completely randomized design.

On 11 Aug. 2001, a recently expanded and exposed leaf was selected from each of six plants in the N fertigation treatment. Net CO₂ assimilation rate was measured midday using a portable gas exchange system (CIRAS-1; PP Systems, Herts, U.K.) at 21.7 °C and saturating light (1700 μmol·m⁻²·s⁻¹) with an ambient CO₂ concentration of 360 μL·L⁻¹.

On 27 Sept. 2001, 60 plants (12 per N fertigation treatment) were sprayed to runoff with a 3% urea solution directed to the foliage (+ fall N), whereas 90 were sprayed with water (- fall N). Individual plastic plates were cut and positioned over the

soil of potted plants to prevent the foliar solution from dripping into the soil.

On 7 Dec. 2001, six plants per N fertigation treatment that received no N in the fall were destructively harvested and separated into leaves, crowns, and roots to assess biomass, carbohydrate, and N reserve status at the end of the growing season (30 plants total). In preparation for the winter, the remaining 120 plants were set into trenches in the field and covered with straw mulch from 3 Dec. 2001 until 13 Mar. 2002, at which time they were removed from the trenches.

In March, six individual plants from each treatment combination were removed from pots and sand was washed off roots with high-pressure tap water. Next, plant material was separated into component tissues (leaf, crown, and roots) and thoroughly rinsed under running tap water. Plant material was dried in a forced-air oven at 100 °C for 60 min and further drying was set at 70 °C for 48 h or until no further changes in weight were observed. Dry weights of roots, crowns and leaves, reserve N, and carbohydrate content were determined. Dry tissues were ground in a Wiley mill (Thomas Scientific, Philadelphia, PA) to pass through a 0.50-mm sieve. Ground samples were stored in desiccators over anhydrous CaCl₂ under vacuum until analyzed. Samples were redried at 40 °C for 8 h before weighing subsamples for N and carbohydrate analysis.

Subsamples of 3.5 to 4.0 mg were weighed into 6 × 4-mm tin cups on a microbalance (AT 20; Mettler-Toledo Columbus, OH) and total N was determined by combustion analysis (NC2100 C/N Analyzer; CE Instruments, Milan, Italy). Carbohydrate was analyzed using the enzymatic microanalytical method with peroxidase/glucose oxidase (PGO) (Trinder, 1969) in 96-well microtiter plates with modifications (Setter et al., 2001) as described subsequently. Assays were done in duplicate and the measurements compared against glucose standards. Soluble sugars were extracted by shaking a 50-mg sample of tissue in 500 μL 80% methanol for 2 h and incubating at 25 °C for 24 h. Extracts were centrifuged at 8160 g_n for 5 min, the supernatant decanted, and the tissue re-extracted twice. Extractions were incubated for 2 h with 150 μL of the methanol mixture. The supernatants were combined and total volume determined.

Glucose and sucrose concentrations were measured by mixing 150 μL of PGO (for glucose) or PGO-invertase (for sucrose) with 45 μL of sample extract. Absorbance was measured at 490 nm before and after 15 min incubation at 30 °C. Glucose and sucrose concentrations were calculated from standard curve linear regression equations. Pre-enzyme absorbance readings were necessary as a result of the pigmentation present in extracts. Tissue pigment absorbance was subtracted from the glucose-generated absorbance signal. Sucrose was determined by subtracting the glucose equivalents in the sample from the total glucose equivalents × 1.9.

Tissue starch concentration was determined by suspending the insoluble fraction from the 80% methanol extraction in 400 μL of water for 4 h at 90 °C. After cooling to room temperature, starch was digested by adding 800 μL Na acetate buffer (45 mM, pH 4.5) containing nine units of high-purity amyloglucosidase [from *Aspergillus niger* van Tieghem (A-7420; Sigma, St. Louis, MO)] and sodium azide. Samples were incubated for 48 h at 40 °C. After incubation, samples were centrifuged (8160 g_n for 5 min) and volume recorded. Debris was taken from some of the samples, stained with iodine (I₂/KI), and observed under a

microscope for changes in color that may indicate incomplete hydrolysis of plant starch to free glucose. Once hydrolysis was deemed to be complete, a 100- μ L aliquot of hydrolysate was diluted with 300 μ L of distilled water. Starch was determined from a 50- μ L aliquot of this solution as described previously for glucose and calculated as $0.9 \times$ the mass of glucose obtained. Total nonstructural carbohydrates were the sum of starch, sucrose, and glucose.

An additional set of 60 plants treated with the five levels of N fertigation were overwintered as described previously, repotted in clean sand, and then half were subjected to supplemental spring N. Fertigation consisted of 10 mM Peters® 20N–8.7P–16.6K with STEM® micronutrients twice per week from 17 May until 17 July 2002. The other half was supplied with a full nutrient solution lacking in N so that plants would be dependent only on their N reserves. These 60 plants, as well as the 60 remaining plants from the fall N study, were maintained in the field through July and total yield per plant was measured during fruiting.

EXPT. 2: INCREASING CARBON RESERVES. On 10 May 2001, 60 plants were fertigated weekly through the irrigation with 7 mM N using Peters® 20N–8.7P–16.6K with STEM® micronutrients during the growing season for 12 weeks. The fertilizer solution pH was 6.6. Flower buds and stolons were removed as described previously.

On 20 Sept. 2001, 20 plants received a 3% foliar urea solution uniformly sprayed to runoff, and the other 40 were sprayed with distilled water. To prevent drippings from the foliar application, the soil was covered with plastic plates until the solution present on leaves had dried.

Six controlled-environment walk-in semiopen polyethylene chambers, located in Ithaca, NY, were randomly assigned to maintain either elevated (1000 μ L·L⁻¹) or ambient (360 μ L·L⁻¹) CO₂ concentrations. Individuals from each set of plants were randomly assigned to the chambers until each chamber had 10 plants with half the total receiving elevated CO₂ and the other half at the ambient level.

Plants remained in chambers for 6 weeks, from 20 Sept. to 5 Nov. 2001. The chambers measured 2.8 $\times$ 3.1 m and were maintained at near outdoor temperature with thermostat-controlled air conditioning units (Kenmore model 79056; Sears, Hoffman Estates, IL) installed inside each chamber. Irradiation was supplied by natural light and similar photosynthetically active radiation conditions were expected inside and outside the chambers as a result of the clear, transparent nature of the polyethylene covering. Small fans within the chambers constantly mixed the air, and the air conditioning units also recirculated air. CO₂ levels inside the chambers were sustained by pulsing in high CO₂ gas (Airgas, Elmira, NY) as needed with solenoid valves controlled by a computerized system (Enviromac; Remote Measurement Systems, Seattle, WA) linked to an infrared gas analyzer (model 422P; NOVA Analytical Systems, Niagara Falls, NY). Gas analyzers were calibrated weekly with CO₂ gas standards (Matheson Trigas, Parsippany, NJ) of known concentrations and the desired concentrations were maintained within 20 NOVA Analytical Systems μ L·L⁻¹ of target settings (Jifon and Wolfe, 2002; Pierce, 2002). Inside the chambers, plants were spaced at 30 $\times$ 20 cm and watered as needed through drip irrigation. Plants were randomized every week within the chamber and among chambers of like treatments and there was no evidence of positional effects.

On 6 Nov. 2001, plants were removed from the chambers and returned to the field at Cornell Orchards. Plants were transferred to trenches built in the field for winter protection. On 15 Mar. 2002, a destructive harvest as described for Expt. 1 was carried out on five plants per each of the four treatment combinations; and carbohydrate analysis, N content, and dry weights were recorded.

The remaining 40 plants were transferred into 7.6-L pots containing new sand and placed outdoors at 40 $\times$ 30-cm spacing on 15 Mar. 2002. Pots were arranged in a completely randomized design. Dead leaves and inflorescences from the previous growing season were removed from all plants and discarded. Fertilization from this point on was either with or without spring N depending on treatment. From 17 May to 17 July 2002, half of the plants were supplied with N twice per week through the irrigation with 10 mM N using Peters® 20N–8.7P–16.6K with STEM® micronutrients. The other half was supplied with a full nutrient solution lacking in N so that plants would be dependent only on their N reserves.

During the runner formation period (summer) in 2002, all runners were removed weekly, counted, and their dry weight obtained. From 13 June to 17 July 2002, fruit was harvested when fully ripe and fruit yield, mean berry count, and weight were determined. Both small and misshapen fruits were included in the harvest. Fruit also was evaluated for color development and soluble solids. Using a color meter (Minolta, Tokyo, Japan), three automatic readings were taken for a random five-berry sample per plant. The means of coefficients *a** and *b** and color luminosity (*L**) were calculated per plant. The percentage of soluble solids was obtained with a digital refractometer (PR-100; ATAGO, Bellevue, WA) for each of the five sampled berries and the mean per plant calculated.

Data were analyzed by considering individual plants within chambers as replications. Although individual plants are not true replications in this case, under such controlled conditions, minimum variation between chambers is expected. Therefore, the factorial design consisted of two levels of CO₂ concentration (elevated and ambient), two levels of fall N treatment (foliar N and control), and two levels of spring N (none and supplemental).

DATA ANALYSES. Analyses of variance were used to identify differences among factors for tissue dry matter and for concentrations and contents of N, glucose, sucrose, starch, total nonstructural carbohydrate (TNSC), and yield using appropriate linear models (SAS Institute, Cary, NC). Pooled SES were calculated.

Results

EXPT. 1: INCREASING NITROGEN RESERVES. Higher first-year fertigation rates resulted in larger plants the second year, supplemental fall foliar fertilizer increased size even more, but supplemental spring N had no effect (Fig. 1). Larger plants had a greater total content of carbohydrate and N (Table 1), but concentrations were more similar across treatments. Roots were more responsive than other plant parts to N fertigation and supplementation, measured as either total N content per plant (Fig. 2) or concentration (Fig. 3). Although crown and leaf N concentration did not respond to N fertigation, crown reserve N fell during the winter, especially at low N fertigation levels (Fig. 3). Crown N was also lower by spring, whereas leaf N was maintained over winter.

Fig. 1. Total strawberry plant dry weight in July 2002 in relation to nitrogen (N) fertilization and foliar urea application in 2001 with no spring applied N (A) or supplemental spring N (B) in 2002. Effect of N fertilization, $P < 0.0001$; N foliar urea, $P < 0.0001$; spring N and interactions were nonsignificant.

Carbohydrates responded in opposite ways to N fertilization. With increasing N reserves, plants exhibited decreased reserve TNSC in roots (Fig. 2), especially in plants also receiving fall foliar urea. Root starch levels were dramatically reduced in response to N fertilization (Fig. 4). Although TNSC content increased with both N fertilization and a foliar urea application (Table 1), this is a reflection of size because plants that received more N were larger. The C/N ratio decreased with increasing N fertilization, although high N plants had more total carbohydrate.

Yield in the fruiting year responded positively to increasing levels of N fertilization in the establishment year (Fig. 5). However, at very high levels of N fertilization, yields either did not respond or decreased in response to fall N supplementation. Highest yields were recorded at moderate N fertilization levels (10 mM) supplemented with foliar urea. Total yield was correlated with total N content, up to ≈ 100 mg/plant, but then no further increase was evident at higher N levels (Fig. 6). Midsummer leaf CO_2 assimilation increased from $\approx 7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 0 mM solution N to $11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 10 mM and remained at this level as N increased to 20 mM (data not shown). Spring N did not contribute to yield, although plant size increased (data not shown).

Yields were a function of fruit numbers rather than size (data not shown). Plants with higher N fertilization tended to fruit later, but by only 2 d ($P < 0.005$), and fruit soluble solids were unaffected. However, fall foliar urea increased fruit soluble solids (6.5% versus 8.7%, $P \leq 0.001$) as well as the chromatic values of a ($P \leq 0.001$), b ($P \leq 0.001$) and L ($P \leq 0.001$).

EXPT. 2: INCREASING CARBON RESERVES. High CO_2 concentrations in fall increased root-to-shoot ratio (1.02 versus 1.45, $P < 0.05$), but total plant dry weight in Mar. 2002 was unaffected (Table 2). However, by July 2002, dry weights of plants and plant parts treated with high CO_2 the previous fall

Table 1. Total starch, glucose, sucrose, total nonstructural carbohydrate (TNSC) and nitrogen (N), and the carbon-to-nitrogen ratio (C/N) in leaves of strawberry plants that received N fertilization only (fertilization) or fertilization + urea applied as a foliar spray in fall (fertilization + foliar urea).^z

N fertilization	Starch	Glucose	Sucrose	TNSC	N	C/N	Dry wt (g/plant)
	(mg/plant)						
Fertilization (mm)							
0	7.2	8.9	17.0	33.1	4.1	37.0	3.3
5	15.7	17.0	28.5	61.2	6.7	37.2	5.3
10	24.1	30.4	65.5	119.9	10.5	35.6	7.9
15	22.7	25.1	39.9	87.7	10.2	38.1	8.3
20	41.8	42.3	54.2	138.3	22.7	35.9	16.1
Fertilization + foliar urea							
0	14.0	14.4	21.7	50.1	9.2	27.7	5.9
5	31.1	24.6	41.5	97.2	32.6	19.7	13.9
10	38.0	37.1	68.7	143.7	39.1	22.0	18.4
15	46.5	48.7	85.9	181.1	44.3	21.7	21.0
20	46.8	43.1	87.8	177.7	55.9	19.1	23.7
Analysis of variance (P values)							
Fertilization	0.0001	0.0001	0.0001	0.0001	0.0001	0.048	0.0001
Foliar	0.0001	0.002	0.001	0.0001	0.0001	0.0001	0.0029
Fertilization $\times$ foliar	0.13	0.10	0.08	NS	0.0001	0.08	0.06

^zPlants were harvested in Mar. 2002. Each value is a mean of six replicates.

NS = nonsignificant.

Fig. 2. Effect of nitrogen (N) fertilization in summer and foliar urea in fall on N and total nonstructural carbohydrate (TNSC) content in strawberry root tissue the next spring (Mar. 2002). Each value is a mean of six plants. Effect of N fertilization, $P \leq 0.0001$; N fertilization + foliar urea, $P \leq 0.0001$; interaction $P \leq 0.0001$.

were higher compared with controls, e.g., 28% for leaves ($P < 0.03$), 17% for crowns ($P < 0.02$), and 21% for whole plants ($P < 0.03$) (Table 2). Yields increased by $\approx 17\%$ ($P < 0.03$) when plants were enriched with CO_2 in fall of the previous year. This was the result of an increase in fruit numbers rather than size.

Although high CO_2 did not significantly affect leaf, crown, or root N concentration (Table 2), the total N content of plants exposed to high CO_2 was increased significantly in all tissues by July 2002 because enriched plants were larger. Similarly, 9 months after application of the foliar urea (July 2002), leaves, crowns, roots, and whole plants were significantly larger than controls. Fall foliar urea application increased yield by $\approx 17\%$ ($P \leq 0.02$), a similar amount to the yield increase from CO_2 enrichment.

A spring N application generally increased N, TNSC, and sugar concentrations of leaves (Table 2), but carbohydrate concentration of roots and crowns was mostly unaffected by spring N. Although plant size increased in response to spring N (50%), yields increased only modestly (12%).

Discussion

Harvesting strawberry fruit removes a significant amount of N from plants (Archbold and MacKown, 1988). Our experiments show that perennial strawberry plants respond to increasing N supply with greater growth and more total N reserve. When plants had a low to moderate N status, increasing the N

Fig. 3. Nitrogen concentrations strawberry plant parts over the winter in relation to nitrogen (N) fertilization during the previous summer. Each value is a mean of six plants. Tissue type $P \leq 0.0001$ in all cases; N fertilization in December and March, $P \leq 0.01$ and 0.0001 , respectively; no significant interactions.

supply in fall through a foliar application resulted in increased yields the next spring. However, there is a limit to the yield increases obtained with N fertilization and supplementation. Yields were not increased when total plant N in spring exceeded ≈ 100 mg/plant. In addition, leaf C assimilation rate reached a maximum at 10 mM N fertilization. In fact, fall urea supplementation in high N plants (those fertiligated with 15 or 20 mM N) resulted in yield reductions. N assimilation requires C units and we measured lower carbohydrate concentrations after N fertilization, especially in roots. It is possible that at very high N levels, the amount of carbohydrates available for fruiting is reduced because of N assimilation demand, leading to reduced yield. Early work on strawberry fertility also demonstrated that high rates of N reduced yield, earliness, and quality (Voth et al., 1967). Ganmore-Neumann and Kafkafi (1983) showed that high rates of ammonium fertilizer solution, coupled with high temperatures, resulted in death of strawberries as a result of depletion of sugars from N assimilation and respiration.

A fall foliar N application decreased the C/N ratio, but this did not negatively impact yield so long as plant N status was not too high. Many studies have examined the impact of foliar N fertilizers on fruit yield (Albregts and Howard, 1986; Ali and

Fig. 4. Strawberry plant starch concentrations over the winter in relation to nitrogen (N) fertilization the previous summer. Each value is a mean of six plants. Effect of tissue type, $P \leq 0.0001$ in all cases; effect of N fertilization in December is nonsignificant (ns), in March $P \leq 0.0001$; no significant interactions.

Lovatt, 1994; Kaska, 1986; Lovatt, 1999; Widders and Hancock, 1994). In many cases, no effects have been measured. However, positive results are most often observed when soil N levels are very low or when the application is made late in the growing season. Our results indicate that a foliar urea application in the fall is effective at increasing yield of strawberry plants if carbohydrate status is adequate and if plants were not heavily fertilized during the growing season.

Spring N applications were not effective at increasing yield significantly in the same year. In one study, spring N did not influence growth or yield, suggesting that sufficient reserve N was present to sustain growth and fruiting through midsummer. In the other, increases were measured, but even when a 50% growth response to spring N was observed, the yield response was only 12%, suggesting that it is mostly fall-assimilated N that is used for flower and fruit development. Spring-applied N influences vegetative plant parts much more than reproductive parts. Applying spring N to compensate for low N reserves is not efficient and can be detrimental to fruit quality (Cooley et al., 1996). Archbold and MacKown (1988) also found that perennial strawberry yield was unaffected by N fertilizer rate and, by using ^{15}N , concluded that N allocated to fruit mostly

Fig. 5. Strawberry yield in relation to previous year's nitrogen (N) treatments. No spring N (A) and 10 mM spring N (B). Each value is the mean of six plants. Effect of N fertilization (A) $P \leq 0.08$; (B) $P \leq 0.008$; effect of N fertilization + foliar urea (A) $P \leq 0.04$; (B) is ns; no significant interactions.

came from reserve N accumulated the previous year. In a second study, Archbold and MacKown (1995) concluded that the strawberry fruit receives more N from storage pools than from newly absorbed N from soil and suggested that soluble reduced N plays a significant role in seasonal storage and internal cycling of remobilized N.

Strawberry plants accumulate biomass in response to CO_2 enrichment (Chen et al., 1997b, 1997c). Under high CO_2 conditions, leaf net assimilation rate increased, leading to enhanced vegetative development (Stitt and Quick, 1989). In our experiment, exposure to elevated CO_2 in the fall resulted in an increase in C allocation to belowground plant parts. This has been observed in other studies (Dyckmans and Flessa, 2002) and is likely the result of plants increasing their sink capacity by developing more storage tissue in roots. In addition, strawberry root activity is known to continue well into the fall, whereas aboveground parts reduce their growth and lose some leaf biomass. Carbohydrates, especially starch, were reallocated from roots and crowns to leaves and fruit from early spring through harvest. The larger root system with its greater C reserves that resulted from CO_2 enrichment led to larger plants and higher yields. Across all treatments, C reserves were correlated with yield, suggesting that C reserves limit strawberry productivity.

To conclude, vegetative growth and yield of strawberries are dependent on both C and N reserves. We found that increasing

Fig. 6. Relationship between strawberry yield and total plant nitrogen or total nonstructural carbohydrate (TNSC) content across all treatments. Each value is a mean of six plants.

C supply in fall increased root size, starch content and concentration, leading to greater yield perhaps by promoting flower bud differentiation in early spring. Yield responses were a reflection of fruit numbers rather than size, and spring N did not have a large effect on yield, further suggesting that it is flower bud differentiation that is affected by C and N reserve status. Plants with higher N reserves also produced greater biomass than those with lower N reserves, but only to a point. At very high N levels, both TNSC and yields were reduced. N content of ≈ 100 mg/plant at harvest was optimal. In our study, stolons were removed periodically because plants were in pots. Typically, in perennial systems, stolons are not removed. This could have affected our results but most likely would have preferentially removed N rather than C because young stolons do not assimilate much C. In the field with stolons intact, plants may be able to use higher levels of N before compromising C reserves.

High N status was achieved by supplementing soil applications with a foliar urea spray during the growing season or with additional spring N, but spring N was not an efficient way to compensate for low N reserves and affect fruiting. The potential exists to economically increase yields by using fall foliar fertilization provided that the plant's carbohydrate status is adequate. This supplementation method can increase reserve N without the environmental risk from soil-applied

Table 2. Cumulative means of starch, sucrose, glucose, and total nonstructural carbohydrate (TNSC) and nitrogen concentration and dry weight in leaves (L), crowns (C), roots (R), or whole strawberry plants (plant) exposed to elevated (+ CO₂) or ambient (- CO₂) carbon dioxide concentrations and treated with urea applied as a foliar spray (- or + N) in the fall or treated with spring N (- or + N) and harvested in Mar. or July 2002.^a

		March 2002										July 2002									
		TNSC (mg/plant)					N (mg g ⁻¹ dry wt)					TNSC (mg/plant)					N (mg g ⁻¹ dry wt)				
		L	C	R	Plant		L	C	R	Plant		L	C	R	Plant		L	C	R	Plant	
CO ₂	-CO ₂	11.1	21.8	24.0	47.5		12.1	12.3	25.0	47.5		26.1	16.6	12.3	10.8		7.0	7.0	7.3	7.3	
	+CO ₂	11.4	18.5	20.7	49.4		10.2	9.2	28.1	49.4		27.5	16.2	12.8	10.4		7.7	7.7	7.5	7.5	
Fall N	-N	11.6	18.0	23.2	45.0		7.9	11.3	25.9	45.0		26.1	15.9	12.2	10.6		6.8	6.8	7.2	7.2	
	+N	10.4	16.7	21.7	52.0		12.3	10.5	29.2	52.0		27.6	16.9	12.9	10.6		7.9	7.9	7.7	7.7	
Spring N	-N											25.2	15.5	12.7	10.0		7.2	6.7	6.7	6.7	
	+N											17.3	12.5	11.3	7.5		8.2	37.2	17.1	56.2	116.3
SE		0.3	1.4	0.7	6.6		1.8	2.0	5.7	6.6		0.6	1.0	0.8	0.3		0.4	0.2	0.2	3.9	5.8
CO ₂		NS	NS	0.003	NS		0.005	0.0002	NS	NS		NS	NS	NS	NS		NS	NS	NS	NS	0.03
Fall N		0.02	0.002	NS	0.07		0.0001	NS	NS	0.07		NS	NS	NS	NS		0.04	0.08	0.03	0.002	0.0002
Spring N												0.001	NS	NS	NS		NS	NS	0.0001	0.02	0.0001
CO ₂ × fall N		NS	0.001	0.02	NS		NS	NS	NS	NS		NS	NS	NS	NS		NS	NS	NS	NS	NS
Fall N × spring N												NS	NS	NS	NS		NS	NS	NS	NS	NS
CO ₂ × fall N × spring N												NS	NS	0.04	NS		NS	NS	NS	NS	NS

^aEach value is a mean of 10 replicates. SE and P values for main effects and interactions are reported (NS = nonsignificant).

fertilization in fall or the detrimental effect on fruit quality from spring fertilization.

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