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Seasonal Patterns of Carbohydrate and Nitrogen Accumulation and Depletion in Strawberry are Affected by Fruiting but Not Day Neutrality

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Abstract

The seasonal patterns of carbohydrate and nitrogen (N) accumulation and depletion have been studied in June-bearing strawberries, but little information is available for day neutral cultivars beyond a single growing season. Non-photoperiodic (day neutral/repeat fruiting) and photoperiodic (short day/June bearing) plants were grown for two years in pots under field conditions. Day neutral plants were either allowed to crop during the first and second growing season or just during the second growing season. Short day plants followed the regular cropping cycle for the perennial system in which plants were deflowered the first year and allowed to crop in the second. Stolons were removed regularly from all plants. Plants were sequentially and destructively harvested and separated into leaves, crowns and roots at several intervals from Sept. 2001 to July 2002. Both photoperiodic types exhibited similar seasonal patterns of carbohydrate and N accumulation and depletion in whole plants and plant parts when they had a similar fruiting status. However, fruiting during the first year in 'Seascape' (day neutral) plants resulted in reduced biomass, second-year yields, starch, and N levels, compared to 'Seascape' and 'Jewel' plants that fruited only in the second year. Day neutral and June bearing cultivars respond similarly to environmental cues regarding carbohydrate and N accumulation, even though the flowering response differs, while first year fruiting in 'Seascape' can negatively impact production capacity in the second year.

Under short photoperiods and the onset of cooler temperatures in autumn, photoperiodic (short day/June bearing) strawberry plants (*Fragaria* x *ananassa* Duch.) accumulate nonstructural carbohydrates and initiate flower buds (Darrow, 1966). The increase in plant carbohydrate in autumn appears to be triggered by short photoperiods and/or cold temperatures (Darnell, 1991; Maas, 1986), which also influence flower bud initiation (Bernier et al., 1993; Bodson, 1977; Durner and Poling, 1988).

The accumulation of N and starch in storage organs in the fall has been correlated with winter survival (Gagnon, Bedard and Desjardins,1990) and productivity the following year (Long, 1935). Macias-Rodriguez, Quero and Lopez (2002) compared carbohydrate differences in short day vs. day neutral cultivars and found differences that they attributed to genotype, but not to day length sensitivity. Eshghi and Tafazoli (2006) found that carbohydrate levels were correlated with induction status, and surmised that carbohydrate content may directly influence induction. Gagnon et al. (1990) measured carbohydrate status in two day neutral and one short day cultivar after various flower removal treatments. They concluded that all cultivars had similar patterns of carbohydrate and N accumulation in the fall, but levels could be influenced by fruiting status. Furthermore, their data showed that temperature and not day length drives the change in carbohydrate accumulation and cold acclimation. However, it is unknown how fruiting and its influence on carbohydrate and N accumulation affect fruiting into a second year since the previous studies were only conducted on first year plants. In many parts of the world, strawberries are grown in perennial systems and fruited multiple years, so the influence of first year fruiting on second year fruiting is important to quantify and understand.

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The objective of this study was to determine if carbohydrate and N accumulation are impacted by photoperiod sensitivity and first-year fruiting, and if so, how this affects fruiting the following year. It is hypothesized that the patterns of carbohydrate and N accumulation in autumn are independent of the photoperiodic response to flowering, and that fruiting in the first year will negatively impact carbohydrate accumulation, N levels and yield the following year.

Materials and Methods

Dormant, single-crowned, certified disease-free plants of 'Jewel' and 'Seascape' were obtained from a commercial nursery (Nourse Farms, Deerfield, Mass.) and selected for uniformity. Bare crowns were planted on 1 May 2001 and each one was 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 x 20 cm at Cornell Orchards, Ithaca, N.Y. Plants were watered as needed with a hand shower to maintain the soil in the pots at or near field capacity. Regular guttation observations in the early hours of the morning were used as an indicator of adequate soil and plant water availability (Glenn and Takeda, 1989). Beginning on 10 May and through mid-Dec. 2001, plants were fertigated twice per week through the irrigation with 10 mM N using Peters® 20-20-20 with micronutrients (STEM®). We estimated that this amount of N would provide approximately 100 $kg \cdot ha^{-1}$ annually if extrapolated to a commercial area basis.

Stolons were removed weekly during the first year according to standard grower practice. Flower buds were removed continuously from all day neutral plants by clipping them from their pedicels during the first 40 days after planting. This was done to facilitate their establishment (Leblanc et al., 1987). Thereafter, half of the plants were allowed to flower and fruit (fruiting plants) during the first (2001) and second growing season (2002) ('Seascape'+ F), as would be done in a regular day neutral type cropping system. Flower bud removal continued in the other half (deblossomed plants) until dormancy, allowing plants to flower and fruit only during the second growing season ('Seascape'-F). Flower buds were removed from all 'Jewel' plants on 21 and 28 May 2001, following the regular cropping system of a June bearing type in which, during the first growing season, plants are deflowered and allowed to crop in the second season. After this initial phase of deflowering, short day plants do not produce additional flowers.

On 3 Dec. 2001, trenches of approximately 15 cm deep were dug in the ground where the strawberry plants were placed, pot-to-pot, over the winter. Plants were covered with 10 cm of wheat straw mulch for winter protection. Mulch remained in place until 13 Mar. 2002, when plants started to show new growth and the straw was raked off the plants and placed into the alleyways. On 1 May 2002, plants were removed from the trench and spaced at 30 x 20 cm. Pots were arranged in a completely randomized design. Dead leaves and inflorescences from the previous growing season were pruned off from all plants and discarded. Plants were fertigated twice per week through the irrigation with 10 mM N using Peters® 20-20-20 with micronutrients (STEM®) from 17 May to 17 July 2002.

Experimental design. The experiment was a factorial design with three levels of plant type ('Jewel', 'Seascape'-F or 'Seascape'+ F) and six whole plant harvest dates (26 Oct. and 21 Nov. 2001, 17 Mar., 17 Apr., 21 May, and 17 July 2002) with six replicates per treatment. At each destructive harvest, individual plants were removed from pots and sand was washed off from 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. Tissues were cut into 1 to 3 cm sections to facilitate drying. Plant material was placed loosely in open and perforated paper bags to allow rapid exposure of moving air into tissues. 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. The initial heat treatment (100°C for 60 min) was used to inactivate carbohydrate-degrading enzymes (Heberer et al., 1985; Hendrix, 1987). Nitrogen losses due to the initial short heat treatment of 100°C for 60 min should have been minimal as nitrate, proteins and amino acids are not volatile. Dry tissues were ground in a mill (Wiley, Thomas Scientific Co., Philadelphia, Penn.) to pass through a 0.50-mm sieve. Ground samples were stored in desiccators over anhydrous $CaCl_2$ under vacuum until analyzed. Samples were re-dried 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 x 4 mm tin cups on a Mettler-Toledo AT20 microbalance and total N was determined by combustion analysis (NC2100 C/N Analyzer, CE Instruments, Milan, Italy).

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 (10,000 rpm, 5 min), the supernatant decanted, and the tissue re-extracted twice. The second and third extractions were incubated for 2 h with 150 µl of the methanol mixture. The supernatants were combined and total volume determined.

Carbohydrate assays were done in duplicate and the measurements were compared against glucose standards. Total carbohydrate was analyzed using an enzymatic micro-analytical method with peroxidase/glucose oxidase (PGO) (Trinder, 1969) with modifications (Setter et al., 2001). Glucose, starch and sucrose concentrations were measured by mixing 150 µl of PGO (for glucose and starch) or PGO-invertase (for sucrose) with 45 µl of sample extract or with 50 µl of the starch hydrolysate. 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 due to 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 times 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*; Sigma A-7420) and sodium azide. Samples were incubated for 48 h at 40°C. After incubation, samples were centrifuged (10,000 rpm, 5 min), and volume recorded. Debris was taken from some of the samples, stained with iodine (I_2/Kl), and observed under a microscope for changes in color that may indicate incomplete hydrolysis of plant starch to free glucose. Then, a 100-µl aliquot of hydrolysate was diluted with 300 µl of distilled water. Starch was determined from a 50-µl aliquot of this solution as described above for glucose and calculated as 0.9 times the mass of glucose obtained. Total non-structural carbohydrates were the sum of starch, sucrose and glucose.

From 13 June to 17 July 2002, fruit was harvested and berry count per plant and total fruit weight per plant were obtained. Both small and misshapen fruits were included in the harvest.

Statistical analyses. Values for tissue and whole plant dry matter (total mass), and for concentrations and total quantities of N, TNSC, glucose, sucrose and starch, were analyzed as a factorial by analysis of variance (SAS, PROC GLM; SAS Institute, Cary, N.C.) to determine the significance of the main effects of plant type, fruiting and plant harvest dates and the respective interactions. Figures and tables are presented with the pooled standard error (SE) of the treatment difference. The pooled standard error was obtained from using the root mean square er-

Table 1. Glucose (Glc) and sucrose (Suc) accumulation in leaves and roots of 'Jewel' and 'Seascape'- F plants from 26 October to 21 November 2001. DW = dry weight.

Sampling Date	'Jewel' Leaves				'Jewel' Roots			
	Glc ($mg \cdot g^{-1}$ DW)	Suc ($mg \cdot g^{-1}$ DW)	Glc (Total mg)	Suc (Total mg)	Glc ($mg \cdot g^{-1}$ DW)	Suc ($mg \cdot g^{-1}$ DW)	Glc (Total mg)	Suc (Total mg)
Oct.	5.7	4.5	357.3	277.3	4.0	9.5	79.5	189.3
Nov.	7.2	6.8	468.8	446.2	4.8	10.6	113.5	249.1
SE	*0.1*	*0.3*	*17.6*	*50.9*	*0.2*	*0.4*	*5.7*	*14.2*
P	*0.0001*	*0.0002*	*0.001*	*0.004*	*0.008*	*NS*	*0.002*	*0.01*

Sampling Date	'Seascape'- F Leaves				'Seascape'- F Roots			
	Glc ($mg \cdot g^{-1}$ DW)	Suc ($mg \cdot g^{-1}$ DW)	Glc (Total mg)	Suc (Total mg)	Glc ($mg \cdot g^{-1}$ DW)	Suc ($mg \cdot g^{-1}$ DW)	Glc (Total mg)	Suc (Total mg)
Oct.	6.5	3.5	253.1	141.3	3.5	6.9	57.5	112.3
Nov.	6.7	6.0	303.3	278.9	4.2	6.9	65.3	107.6
SE	*0.3*	*0.2*	*18.2*	*23.4*	*0.12*	*0.4*	*2.9*	*7.7*
P	*NS*	*0.0001*	*0.08*	*0.002*	*0.009*	*NS*	0.08	NS

ror from the analysis of variance and divided by the root square of the number of replicates per treatment.

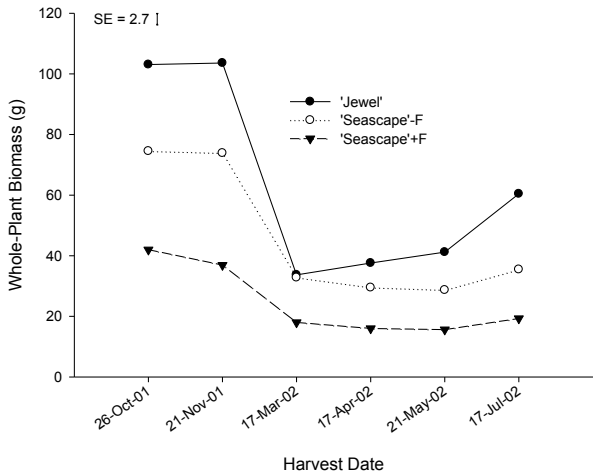

Figure 1. Whole-plant dry weight for 'Jewel', 'Seascape'+ F, and 'Seascape'- F. Each value is a mean of six plants. Treatment $P \leq 0.0001$; harvest date $P \leq 0.0001$; treatment x harvest date is NS.

Results and Discussion

The removal of stolons during the first year would have reduced total potential plant biomass, N and carbohydrate levels, and this could have affected subsequent allocation patterns. However, standard grower practice is to regularly remove stolons, and day neutrals are most often grown in plasticulture systems where stolons are prevented from rooting. Plants with stolons removed are more similar to plants under cultivated conditions.

Plant biomass in both cultivars declined during winter (Fig. 1), regardless of treatment. This was mostly due to a reduction in leaf biomass. However, root growth continued into the winter months even when plants were covered with straw and no photosynthesis was occurring, suggesting that strawberry roots could be acting as storage organs

(data not shown) for reallocated nutrients from leaves. A similar pattern of reallocation has been documented for strawberries in annual plasticulture systems (Fernandez et al., 2001). The mean whole-plant dry weight of 'Seascape'+ F plants was significantly lower than that of plants in the other two treatments that did not fruit the first year, and the difference in plant size was maintained through spring (Fig. 1). 'Jewel' in particular, exhibited greater growth in spring. This suggests that fruiting in the first year significantly reduced growth capacity in the subsequent year.

Total non-structural carbohydrates accumulated in the fall (Oct.-Nov.) in leaves and roots of 'Jewel', as well as leaves of 'Seascape'- F plants, but no increase was observed in 'Seascape'+ F plants (Figs. 2 and 3). In general, glucose and sucrose concentrations and content also increased in the fall (from Oct.-Nov. 2001) in leaves and roots of both 'Jewel' and 'Seascape'- F plants (Table 1), but no increase in sugar accumulation was observed in 'Seascape' + F plants (data not shown). Fruiting in the planting year negatively impacted the ability of 'Seascape' to accumulate carbohydrate (both sugar and starch) prior to winter.

In late winter to early spring (Mar.-Apr.), leaf TNSC declined progressively to near zero in all treatments (Fig. 2). Root TNSC also declined steadily from Nov. through July. Once above-ground growth began in spring, carbohydrates in leaves and crowns were replenished with the development of new leaves. These results are in agreement with the general seasonal pattern of reserve carbohydrate concentrations found in deciduous crops (Hennerty and Forshey, 1971) in which a decrease of reserve carbohydrate occurs over the winter and when initial growth takes place in the spring. Replenishment of reserve carbohydrates develops gradually as more leaves become C exporters rather than sinks. Then, a rapid accumulation of reserves takes place once plants have reduced their growth, or after harvest, reaching maximum levels in

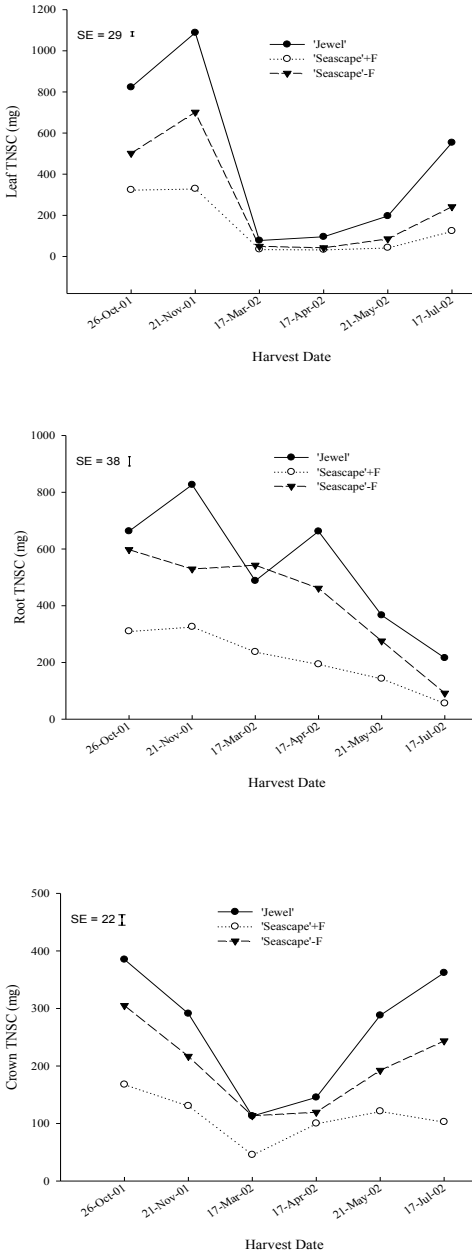

Figure 2. Total non-structural carbohydrates (mg) in leaves, crowns and roots of 'Jewel', 'Seascape'+ F, and 'Seascape'- F plants. Each value is a mean of six replicates. Treatment and harvest date effects are significant at $P < 0.001$.

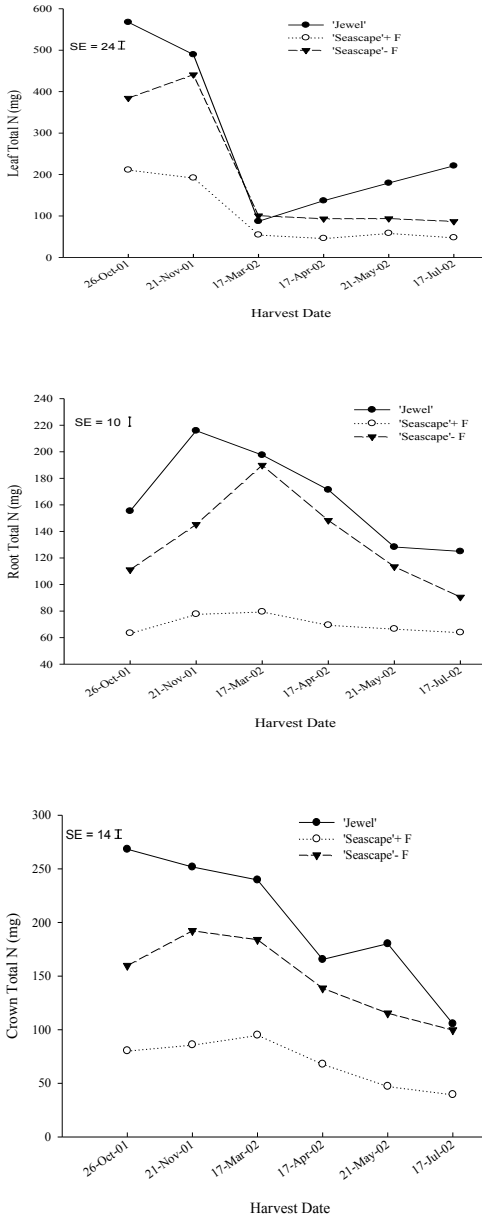

Figure 3. Total nitrogen (mg) in leaves, crowns and roots of 'Jewel', 'Seascape'+ F, and 'Seascape'- F plants. Each value is a mean of six replicates. Treatment and harvest date effects are significant at P< 0.001.

the fall. In our work, the continued depletion of root carbohydrate in the spring is likely the result of rapid leaf and fruit development before leaves have completely expanded and are operating at full photosynthetic capacity. In addition to carbohydrate depletion due to respiration and maintenance during winter, carbon depletion also has been attributed to the high rate of nutrient uptake known to occur in spring (Buwalda and Smith, 1990). N uptake, in particular, can cause a reduction in carbohydrate levels (Acuña-Maldonado and Pritts, 2009) since most N-based compounds require a carbon skeleton. Although some of the N that accumulated in roots during the winter would likely have come from remobilization from leaves, some could have come from root uptake, promoting the continued decline of root carbohydrate.

Fruiting may have negatively affected carbohydrate reserves in roots, but TNSC levels in leaves and crowns increased even while the plants were fruiting. N levels, in contrast, continued to decrease during fruiting in roots and crowns (Fig. 3). Only in leaves of 'Jewel' did N levels increase slightly during the fruiting period. The negative impact of fruiting on the ability of strawberries to acquire N has been reported elsewhere (Archbold and MacKown, 1995).

The contrast between patterns of N and TNSC is most dramatic when expressed as concentration rather than total dry weight per plant. Since total plant biomass decreased more rapidly in autumn and increased more rapidly in spring than total N, when expressed as tissue concentration, the seasonal pattern of N accumulation and depletion exhibited a pattern that was inverse to that of starch (Figs. 4 and 5). For example, starch concentration declined during winter (Nov. through Mar.) as expected when photosynthesis ceases, whereas N concentration increased during this time. When starch concentration began to increase with the onset of photosynthesis, N concentration began to decrease. The overall seasonal patterns of accumulation and depletion for N and starch were similar among cultivars,

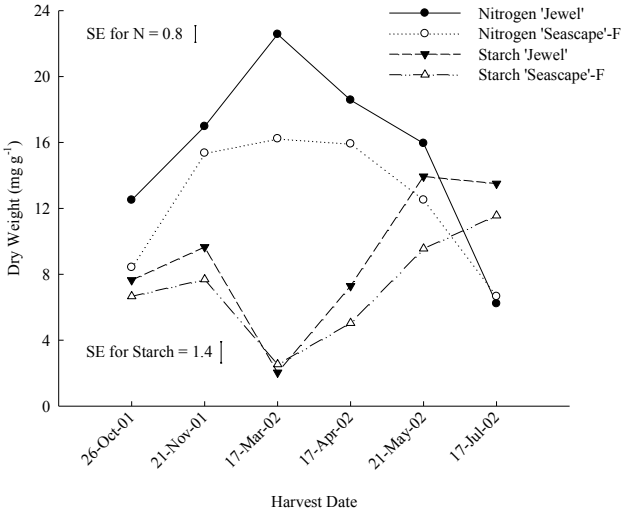

Figure 4. Nitrogen and starch concentrations (mg·g^{-1} dry weight) of crown tissue in 'Jewel' and 'Seascape'- F plants. Each value is a mean of six replicates. Treatment (nitrogen) $P \leq 0.0001$; treatment (starch) $P \leq 0.05$; harvest date (nitrogen) $P \leq 0.0001$; harvest date (starch) $P \leq 0.0001$; treatment x harvest date (nitrogen) $P \leq 0.001$; treatment x harvest date (starch) is NS.

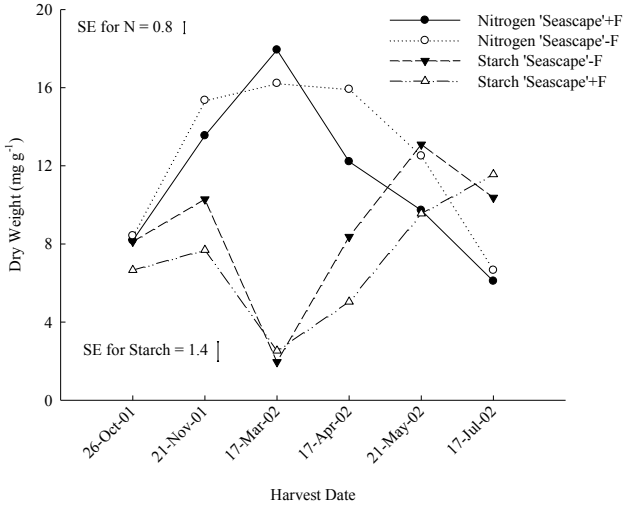

Figure 5. Nitrogen and starch concentrations (mg·g^{-1} dry weight) of crown tissue in 'Seascape'+ F and 'Seascape'- F. Each value is a mean of six replicates. Treatment (nitrogen) $P \leq 0.0001$; treatment (starch) $P \leq 0.05$; harvest date (nitrogen) $P \leq 0.0001$; harvest date (starch) $P \leq 0.0001$; treatment x harvest date (nitrogen) $P \leq 0.001$; treatment x harvest date (starch) is NS.

indicating that they are not affected by photoperiodic type.

'Seascape'+ F plants were more affected by an early spring frost (on 7 Apr. 2002) than the other cultivars that underwent only vegetative growth during the first season. Freeze damage was assessed by counting the total number of flower buds and black flowers three days after the freeze. The percent of blackened flowers at that time was 14%, 48% and 59% for 'Jewel', 'Seascape'- F, and 'Seascape'+ F, respectively. Day neutrals have been reported to be less hardy than June bearing cultivars (Gagnon et al., 1990) and may be particularly susceptible to freeze injury when carbohydrate levels are low. With the return of warmer weather, plants continued their growth and showed no further signs of injury.

'Jewel' had the highest yield of the three plant types in the second year and 'Seascape'-F had significantly higher yields than 'Seascape'+ F (Fig. 6). The higher yield and biomass accumulation observed in plants that fruited only in the second growing season is likely to be the result of compensatory development in response to reduced competition for assimilates and the concomitant accumulation of reserves for the subsequent year's growth (Swartz et al., 1988). These results are consistent with reports where continued flower bud removal was positively associated with second-year

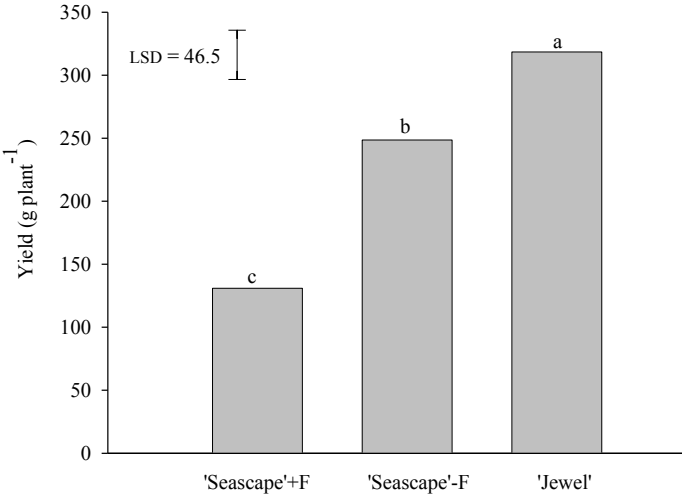

Figure 6. Yield (g·plant^{-1}) of 'Jewel' and 'Seascape' plants in 2002 (second year). Each value is a mean of six plants. Mean separation by Fisher's LSD test ($\alpha = 0.05$).

yields (Pritts and Worden, 1988) in day neutrals. A delay in fruit maturation sometimes is observed in plants exhibiting compensatory responses (English-Loeb et al., 1999), but flower bud removal in 'Seascape'- F did not result in delayed maturation in the second year ($P \leq 0.12$).

Although marked differences exist between the two types of strawberry, they were comparable in their N and carbohydrate partitioning patterns and relative pool sizes when treated similarly. Both photoperiodic types increased starch and N concentration with the onset of winter (Oct. – Nov.), both depleted carbohydrates during winter while increasing the N concentration, and both replenished carbohydrates with the onset of photosynthesis in spring. However, fruiting during the first growing season negatively impacted the total amount of accumulated N, sugar and starch, and fruiting during the second growing season also appeared to negatively affect the plant's ability to accumulate N. Even though plants were actively growing and fruiting during the second growing season, total N concentration declined.

Growers of perennial strawberries must learn to manage first year fruiting in day neutral cultivars in a way that takes advantage of higher prices later in the season of the first year while minimizing impacts on second year fruiting potential. Our data suggest that increasing N levels in the spring of the second year might increase yield since N levels were particularly low in plants that had previously fruited. While increasing the supply of N appears to be a route for increasing yield in the second year, earlier work suggests that late fall is a better time to apply supplemental N than spring (Acuña-Maldonado and Pritts, 2009) when it is more efficiently used for fruiting.

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