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Full Length Research Paper

Late-season nitrogen applications in high-latitude strawberry nurseries improve transplant production pattern in warm regions

Daniel S. Kirschbaum[1]*, Kirk D. Larson[2], Steve A. Weinbaum[2] and Theodore M. DeJong[2]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA). EEA Famaillá, Argentina.
[2]Department of Plant Sciences, University of California Davis, CA, USA.

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The influence of late-season nitrogen (N) applications on the fruiting pattern of strawberry runner plants of 'Camarosa' was determined over three growing seasons. Experiments were carried out in high-latitude nurseries in northern California and fruit production trials were established in southern California. A total of 80 kg/ha of foliar nitrogen was delivered in three applications to the nursery in late summer. Late-season foliar nitrogen applications: (1) increased early yields (+22% on average) as well as the number of early marketable fruit, (2) did not affect total season yields, fruit size, appearance and firmness and (3) resulted in greater N concentration in leaves, crowns and roots. Runner plants with leaf N concentration within the sufficiency range (1.9 - 2.8% of dry mass) produced the highest early yields. Total nonstructural carbohydrate concentrations decreased in most of the N-treated plants. Apparently, nursery late-season foliar nitrogen applications enhance N mobilization to crown and root, stimulate plant activity during the period of flower differentiation after planting, accelerating flower development and contributing to the advancement of fruit production.

Key words: *Fragaria* x *ananassa* Duch, foliar urea, carbohydrates, high-latitude nursery, plant maturity.

INTRODUCTION

Nitrogen is essential during early growth, bud differentiation and flowering in strawberry (Albregts and Howard, 1980). However, excess N nutrition can result in excessive foliage, increased susceptibility to pathogens, poor performance and survival after transplant, fruit softening and late ripening (May and Pritts, 1990; Strand, 1994). Nitrogen and total nonstructural carbohydrate (TNC) translocation to storage tissues, such as roots and crowns, in high-latitude (HL) locations occurs simultaneously in the fall (Gagnon et al., 1990; Kirschbaum, 2005; Long, 1935).

The role of N reserves on fruiting patterns has not been studied in strawberry runner plants propagated in California HL nurseries, in comparison with other exhibit seasonal fluctuations in N content, with peak levels in November (northern hemisphere) when nearly production systems and environments. In multiple-year (biennial or perennial) strawberry crop systems, plants 80% of the total N is in the leaves. After this peak, leaf N decreases to ~1% in late autumn and winter as a result of leaf senescence and death. Simultaneously, N content increases in roots (mainly) and crowns, which serve as N storage organs in winter. Early in spring, N stored in root and crown tissues is mobilized during development of buds and new leaves (Long and Murneek, 1937; Mann, 1930).

Reductions in TNC content in roots of strawberry runner plants occurred in Germany when the rate of N applied in the nursery in late August was increased from 40 to 80 kg/ha (Kreusel and Lenz, 1996). In grape (*Vitis vinifera* L.) vines, reductions in plant TNC levels following foliar N applications was found to be nearly equivalent to the carbon compounds incorporated into proteins and

*Corresponding author. E-mail: dkirschb@correo.inta.gov.ar. Tel: +54-3863461048. Fax: +54-3863461546.

amino acids, indicating that plants use TNC reserves for the biosynthesis of amino acids and proteins as a mechanism to store N and C (Xia and Cheng, 2004). However, the specific interrelations between TNC and N during the storage process are not well known in strawberry runner plants.

Studies conducted in Guelph (43°32'N, Canada) suggest that enhanced runner plant growth during the period of flower initiation (August - September) maximizes yield in the fruiting field. In other words, strawberry yield potential is determined during the period of flower bud differentiation (Strik and Proctor, 1988), which takes place in late summer and early autumn. When N is withheld until the middle of September, there is a reduction of the number of flowers (Long, 1939). In California, HL strawberry nursery managers usually terminate N applications by the beginning of August in an effort to "harden off" developing runner plants. Clearly, there is a contradiction between the principle supporting current practices and the physiological concepts addressed by researchers (Strik and Proctor, 1988; Long, 1939).

In general, TNC reserves are considered to play a major role in determining strawberry transplant vigor but N reserves have largely been overlooked. Therefore, the objectives of this study were to determine the effects of late summer and early fall foliar N applications in high-latitude nurseries, on the fruiting pattern of fresh-dug plants.

MATERIALS AND METHODS

Nursery experiments

Nursery treatments were applied in a commercial runner plant propagation nursery near Dorris, CA (41°58'N, 121°55'W, 1292 m elevation) in 2002, 2003 and 2004 and in Lake Shastina, CA (41°30'N, 122 °23'W, 862 m elevation) in 2003. Cold-stored 'Camarosa' mother plants were used as nursery stock. In 2002, plants were established on April 20 in offset double rows with 60-cm-in-row plant spacing. In 2003, plants were established on 45-cm-in-row plant spacing on April 4. In 2004, plants were established on 45-cm-in-row plant spacing on April 14. Water was supplied by sprinkler irrigation in 2002 and by drip irrigation in 2003 and 2004. Plants were grown with standard commercial nursery management.

Prior to foliar N applications, every year the nursery was fertilized with 225 kg N/ha, 282 kg P_2O_5/ha and 296 kg K_2O/ha. Other nutrients were applied at lower rates also according to standard fertilization practices for nurseries in the region.

Every year, the first week of August, a homogeneous 500 m^2 plot was selected in the nursery and divided in two sections of 250 m^2. One of them was treated with foliar N and the other was sprayed with water (control). Nitrogen treatments consisted of a total dose of 80 kg/ha of foliar N, distributed in three applications. In 2002, foliar urea (0.5% concentration) was delivered at the following dates and rates: August 29, 7.6 kg N/ha; September 5, 15.2 kg N/ha and September 20, 56.0 kg/ha. In 2003, foliar urea (0.5% concentration) was delivered in three applications of ~27 kg N/ha each, on Aug 15, Aug 30 and Sep 15. In 2004, instead of urea, UAN 32 (1.0% concentration) was used as N source, which was delivered in three applications of ~27 kg N/ha each, on Sep 6, Sep 13 and Sep 20.

First and second daughter plants (the first and second runner plants that develop in a series from the stolons of the mother plants) were randomly harvested from treated and control plots the first week of October (7 Oct 2002, 4 Oct 2003 and 1 Oct 2004) and cold stored at 1°C for 2 - 4 days. A group of plants was separated for laboratory analyses and another group of plants was planted in the field for fruiting experiments, in southern California.

From the first group of plants, samples of 10 - 20 plants of each runner order and nursery N treatment were washed thoroughly, subjected to leaf area measurements and dried at 65°C for 4 days. In 2002 and 2004, after drying and dry mass determination, 5 - 10 plants were analyzed for soluble (reducing sugars) and non-soluble (starch) TNC and total N. The analytical procedure for TNC consisted of enzymatic starch hydrolysis with amyloglucosidase, followed by high performance liquid chromatography (HPLC) for analysis of reducing sugars (Smith, 1969). Total N analysis was performed with a Carlo Erba (Italy) elemental analyzer.

Fruiting field experiments

The second group of plants was planted in experimental plots at the University of California's South Coast Research and Extension Center, Irvine (33°39'N, 117°41'W) 3 - 4 days after they were harvested from the nursery. Leaves were removed prior to planting. Preplant soil fumigation was applied using a (wt:wt) mixture of 2 methyl bromide : 1 chloropicrin at a rate of 392 kg/ha. Plants were established in offset 4-row beds 162 cm wide x 40 cm high using a 40 cm in-row plant spacing (61,250 plants per hectare), with 10 plant plots of a unique daughter order and N treatment. The experimental setup was a completely randomized design with 3 replications. All plots were maintained according to recommendations for California commercial winter plantings (Welch, 1989).

Fruit were harvested weekly, the first season from 10-Dec-2002 to 21-May-2003; the second season from 2-Jan to 25-May-2004 and the third season from 21-Dec-2004 to 11-Apr-2005, according to commercial fruit maturity standards. Fruit yields were determined for each plot on a per-plant basis. In 2004, the number of flowers per plant was determined 37 days after planting in Irvine (11 November, 2004). Data were subjected to analysis of variance and means were separated using SAS (SAS Institute, 2003).

RESULTS AND DISCUSSION

Late-season N applications increased early yields (Table 1) and the number of early marketable fruit (Table 2). On average, total early yield per plant increased by 22%, within a range of 10 to 56%, in contrast with studies conducted in perennial strawberry growing systems, in New York, or in strawberry nurseries in Mexico, where fall applied urea had no impact in fruit production patterns (Carrillo-Mendoza et al., 2005; Acuña-Maldonado and Pritts, 2008).

In 2004, late-season foliar N applications increased the number of flowers per plant, 37 d after planting ($P = 0.0018$, data not shown). Fruit characteristics such as size (expressed as average marketable fruit weight), appearance and firmness were not affected by late-season N applications (Table 2). There was no effect of foliar N applications on total seasonal yields.

The results suggest that early strawberry fruit production is enhanced by foliar N fertilization near the end of the nursery runner propagation period, in agreement with

Table 1. Effects of late season nursery N applications (N0=control; N1=foliar N) on total and early season yields of 'Camarosa' daughter 1 (D1) and daughter 2 (D2) plants, dug from California high-latitude nurseries (Dorris, DR: 2002, 2003 and 2004; Lake Shastina, LS: 2003). Fruit production plots were established in Irvine, California.

Year	Daughter	Total season yield (TSY) (g/plant)^z		Early season yield (ESY) (g/plant)^y		Early season marketable fruit number per plant (EMF)	
		N0	N1	N0	N1	N0	N1
2002	D1	1300	1327	394	433	9.7	10.0
	D2	1277	1394	348	415	7.8	9.3
2003 DR	D1	1554	1673	211	255	5.0	6.3
	D2	1310	1369	152	179	3.1	4.3
2003 LS	D1	1362	1446	212	331	4.8	7.3
	D2	1349	1323	169	193	4.0	4.1
2004	D1	877	935	297	350	6.7	7.1
	D2	818	873	284	337	5.9	7.3
Analysis of variance		**TSY**		**ESY**		**EMF**	
2002							
Nitrogen (N)		ns		***		*	
Daughter (D)		ns		*		***	
N x D		ns		Ns		ns	
2003							
Nitrogen (N)		ns		*		*	
Daughter (D)		ns		**		***	
Nursery		ns		ns		ns	
N x D		ns		ns		ns	
2004							
Nitrogen (N)		ns		*		ns	
Daughter (D)		ns		ns		ns	
N x D		ns		ns		ns	

^{z} Last harvests: 21-May-2003, 25-May-2004 and 11-Apr-2005. ^{y} Last harvests: Mar 1st.
Analysis of variance: *, **, *** and ns, signifcant at $p < 0.05$, 0.01, 0.001 and non significant, respectively.

previous reports (Kreusel and Lenz, 1996; Rodgers et al., 1985), which show that N applications increase fruit production but also alter TNC concentration in plant tissues. Late-season nursery N applications did not affect plant biomass significantly (Table 3). Leaf area (LA) and number of leaves per plant were not significantly influenced by treatments (data not shown).

Plants receiving late-season N in HL/HE nurseries had higher N concentration in all plant tissues (Table 4). N-treated plants produced the highest early yield, suggesting that N was stored in roots and crowns and then it was reutilized when runner plants were established for fruit production in a warmer region. Consequently, the present study is showing that N plays a major role in early plant growth and flower/fruit development in strawberry, not only in perennial or biennial growing systems (Acuña-Maldonado and Pritts, 2008; Tagliavini et al., 2005), but also un annual systems, where the nutritional status of the runner plant (tightly linked to the fertilization program applied in the nursery) has a tremendous impact in the fruiting pattern.

Late-season N applications, daughter plant order and type of plant tissue affected plant N concentration and partitioning (Table 4). Late-season N applications significantly increased N concentration and decreased TNC concentration in leaves, crowns and roots. Foliar applied N late in summer or early in autumn follows the natural process of nutrient translocation from leaves to storage organs occurring in the plant at that time of the year. Nonstructural carbohydrate and N translocation to storage tissues occur simultaneously in the fall and there is a strong correlation between cold tolerance acquisition and accumulation of TNC and N in roots, suggesting that both TNC and N concentrations increase concurrently with decreasing temperatures (Gagnon et al., 1990).

Total nonstructural carbohydrate concentration in strawberry runner plants is positively correlated with exposure to cold temperatures and short photoperiods (Kirschbaum, 2005; Kirschbaum et al., 1998; Ruan et al., 2009). In this study, root concentration of TNC was negatively correlated with N applications. In addition to chilling hour accumulation, N availability in the fall seems

Table 2. Effects of late season nursery N applications on early season fruit quality of 'Camarosa' daughter 1 (D1) and daughter 2 (D2) plants, dug from California high-latitude nurseries (Dorris, DR: 2002, 2003 and 2004; Lake Shastina, LS: 2003). Fruit production plots were established in Irvine, California.

Year	Daughter	Average marketable fruit weight (AFW) (g)		Appearance[z] (APP)		Firmness[y] (FMN)	
		N0	N1	N0	N1	N0	N1
2002	D1	35.8	37.0	3.1	3.2	3.7	3.7
	D2	39.2	39.3	2.8	3.0	3.6	3.7
2003 DR	D1	31.9	33.7	2.8	3.1	3.8	4.0
	D2	36.8	34.0	3.0	2.9	3.7	3.9
2003 LS	D1	33.7	36.0	2.8	2.7	3.8	3.6
	D2	34.5	35.7	2.6	2.9	3.9	3.9
2004	D1	31.7	32.8	2.8	2.8	3.8	3.9
	D2	33.9	31.9	2.9	2.8	3.9	3.7
Analysis of variance		**AFW**		**APP**		**FMN**	
2002							
Nitrogen (N)		ns		ns		ns	
Daughter (D)		***		***		ns	
N x D		ns		ns		ns	
2003							
Nitrogen (N)		ns		ns		ns	
Daughter (D)		ns		ns		ns	
Nursery		ns		*		ns	
N x D		ns		ns		ns	
2004							
Nitrogen (N)		ns		ns		ns	
Daughter (D)		ns		ns		ns	
N x D		ns		ns		ns	

[z, y]Fruit appearance and firmness scores: 5 = best.
Analysis of variance: *, **, *** and ns, signifcant at $p < 0.05$, 0.01, 0.001 and non significant.

Table 3. Effects of late season N applications (N0 = control; N1 = foliar N) on the dry mass of 'Camarosa' daughter 1 (D1) and daughter 2 (D2) plants, dug from California high-latitude nurseries (Dorris, DR: 2002, 2003 and 2004; Lake Shastina, LS: 2003).

Year	Daughter	Plant dry mass (PDM) (g)	
		N0	N1
2002	D1	8.22	9.73
	D2	4.41	6.21
2003 DR	D1	5.48	7.25
	D2	2.58	2.24
2003 LS	D1	9.90	8.48
2004	D1	9.16	8.56
	D2	6.60	7.47

to be a major factor controlling carbohydrate partitioning and accumulation in HL/HE nurseries. The role of the crown as N-reserve organ appears to be secondary to that of roots, as previously observed with TNC. Compared with roots, crowns have a smaller biomass (Larson and Shaw, 1996), which limits their capacity as reserve organs.

Control plants foliage was light-green to yellowish-green in the late summer possibly indicating N deficiency, while leaves of N-treated plants remained dark green

Table 3. Continued.

Analysis of variance	PDM
2002	
Nitrogen (N)	ns
Daughter (D)	*
N x D	ns
2003	
Nitrogen (N)	ns
Daughter (D)	***
Nursery	***
N x D	ns
2004	
Nitrogen (N)	ns
Daughter (D)	***
N x D	ns

Figure 1. Effects of late summer and early fall foliar N applications on ‘Camarosa’ cultivar, in a high-latitude/high-elevation nursery in Siskiyou County, California, USA. N0 = without foliar N (control); N1= with foliar N.

until plants were dug (Figure 1). Leaves of control plants were N-deficient (Table 4) according to the standard sufficiency range for total N in strawberry leaf blades, in which 1.9, 2.0 - 2.8 and 4.0% (on a dry weight basis) are considered “deficient”, “sufficient” and “excess”, respectively (Pritts and Handley, 1998). Late-season N applications resulted in sufficient leaf N concentration and this apparently improved plant vigor and fruiting patterns.

These results suggest that late-season N applications allow plants to remain actively growing during the period of flower differentiation, enhance N mobilization to crown and root, increase the number of flowers early in the season ($P > 0.0018$) and contribute to greater early-season fruit production. Plants treated with N did not exhibit excessive vegetative growth, transplant stress or susceptibility to diseases.

Nitrogen reserves are major resources for fall fresh-dug runner plants. An almost century-old concept that confers a major role to TNC over any other reserve nutrients needs to be re-examined. Nitrogen reserves have largely been overlooked as having a major role in plant establishment and early fruit development. The N cycling

Table 4. Effects of nursery late-season N applications (N0 = control; N1 = foliar N) on the concentration of N-total and total nonstructural carbohydrates (TNC) in leaflet, crown and root of 'Camarosa' daughter 1 (D1) and 2 (D2) plants.

Year	Daughter	Leaflet		Crown		Root	
		N0	N1	N0	N1	N0	N1
Total nitrogen concentration (N) (% DM)							
2002	D1	1.66	2.29	1.30	1.73	1.19	1.33
	D2	1.72	1.84	1.16	1.54	1.02	1.02
2004	D1	1.68	2.81	0.80	1.80	0.86	2.02
	D2	1.47	1.95	0.69	1.11	0.75	1.01
TNC concentration (% DM)							
2002	D1	6.40	4.85	10.45	9.03	11.04	7.40
	D2	6.15	4.75	9.67	9.40	12.93	10.28
2004	D1	7.63	7.23	9.63	7.33	8.87	8.47
	D2	9.97	8.33	9.93	8.80	10.90	6.83
Analysis of variance		**N**			**TNC**		
2002							
Nitrogen (N)		**			*		
Daughter (D)		*			ns		
Tissue (T)		***			***		
2004							
Nitrogen (N)		***			***		
Daughter (D)		***			*		
Tissue (T)		***			ns		
NxD		***			ns		
NxDxT		ns			*		

Analysis of variance: *, **, *** and ns, signifcant at $p < 0.05$, 0.01, 0.001 and non significant, respectively. Statistically non-significant interactions are not shown.

process in strawberries remains largely unknown. Which amino acids and proteins constitute the N reservoir in storage tissues of strawberry plants? Vegetative storage proteins play an important role in nitrogen storage and are commonly bioactive in herbaceous plants and a few have been also identified in woody plants (Tian et al., 2007). Further research will lead to determine if reserve proteins and reserve amino acids in strawberry are the same of those found in other species.

Our results demonstrate the importance of N nutrition in late stages of strawberry nurseries and raise new questions related to N management in nurseries, rates, methods of application (soil, irrigation, foliar) and application timing. Challenging long-standing dogma, our data indicate that late season foliar N applications are desirable when leaf total N is below the sufficiency range, in nurseries programmed to produce fresh-dug plants in early autumn. Research should be conducted to adjust these results to other cultivars and environments.

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REFERENCES

Acuña-Maldonado LE, Pritts MP (2008). Carbon and Nitrogen Reserves in Perennial Strawberry Affect Plant Growth and Yield. J. Amer. Soc. Hort. Sci. 133: 735-742.

Albregts EE, Howard CM (1980). Accumulation of nutrients by strawberry plants and fruit grown in annual hill culture. J. Amer. Soc. Hort. Sci. 105: 386-388.

Carrillo-Mendoza O, Rodríguez-Alcázar J, Cano-Medrano R, López-Jiménez A (2005). Foliar application of urea and sucrose, their effects on nursery plant conditioning and production of strawberry (*Fragaria × ananassa* Duch.) 'CP 99-3A'. Agrociencia. 39: 195-204.

Gagnon B, Desjardins Y, Bedard R (1990). Fruiting as a factor in accumulation of carbohydrates and nitrogen and in fall cold hardening of day-neutral strawberry roots. J. Amer. Soc. Hort. Sci. 115: 520-525.

Kirschbaum DS (2005). Biomass and total nonstructural carbohydrate (TNC) partitioning in relation to nursery digging date, and subsequent growth, development and fruiting pattern of strawberry (*Fragaria x ananassa* Duch) runner plants propagated in California high-latitude nurseries. Ph.D. Dissertation, University of California, Davis, USA, p. 129.

Kirschbaum DS, Cantliffe DJ, Darnell RL, Bish EB, Chandler CK (1998). Propagation site latitude influences initial carbohydrate concentration, partitioning, growth, and fruiting of 'Sweet Charlie' strawberry transplants grown in Florida. Proc. Fla. State Hort. Soc. 111: 93-96.

Kreusel U, Lenz F (1996). Influence of N fertilization on the distribution of carbohydrates in shoots and roots of strawberry runner plants. Erwerbsobstbau 38: 179-183.

Larson KD, Shaw DV (1996). Soil fumigation, fruit production, and dry matter partitioning of field-grown strawberry plants. J. Amer. Soc. Hort. Sci. 12:1137-1140.

Long JH (1935). Seasonal changes in nitrogen and carbohydrate content of the strawberry plant. Proc. Amer. Soc. Hort. Sci. 33: 386-388.

Long JH (1939). The use of certain nutrient elements at the time of flower formation in the strawberry. Proc. Amer. Soc. Hort. Sci. 37: 553-556.

Long JH, Murneek AE (1937). Nitrogen and carbohydrate content of the strawberry plant, seasonal changes and the effect of fertilizers. Re. Bull. Mo. Agric. Exp. Stn. p. 252.

Mann CET (1930). Studies in the root and shoot growth of the strawberry. V. The origin, development, and function of the roots of the cultivated strawberry (*Fragaria virginiana x chiloensis*). Ann. Bot. 44: 56-84.

May G, Pritts MP (1990). Strawberry nutrition. Adv. Strawberry Prod. 9:10-24.

Pritts MP, Handley DT (1998). Strawberry Production Guide for the Northeast, Midwest, and Eastern Canada (NRAES-88), New York, USA.

Rodgers CO, Izsak E, Kafkafi U, Izhar S (1985). Nitrogen rates in strawberry nursery on growth and yield in the field. J. Plant Nutr. 8:147-162.

Ruan J, Yoon CS, Yeoung YR, Larson KD, Ponce L (2009). Efficacy of highland production of strawberry transplants Afr. J. Biotechnol. 8: 1497-1501.

SAS Institute (2003). SAS 9.1. SAS Institute Inc., Cary, NC, USA.

Smith D (1969). Removing and analyzing total nonstructural carbohydrates from plant tissue. Wi. Agric. Exp. Stn. Res. Rep. p. 41.

Strand LL (1994). Integrated pest management for strawberries. Publ. 3351. University of California, Division of Agriculture and Natural Resources. Berkeley, California, USA, p. 142.

Strik BC, Proctor JT (1988). The importance of growth during flower bud differentiation to maximizing yield in strawberry genotypes. Fruit Var. J. 42: 45-48.

Tagliavini M, Baldi E, Lucchi P, Antonelli M, Sorrenti G, Baruzzi G, Faedi W (2005). Dynamics of nutrients uptake by strawberry plants (*Fragaria* × a*nanassa* Duch.) grown in soil and soilless culture. Eur. J. Agron. 23:15-25.

Tian WM, Peng SQ, Wang XC, Shi MJ, Chen YY, Hu ZH (2007). Vegetative storage protein in *Litchi chinensis*, a subtropical evergreen fruit tree, possesses trypsin inhibitor activity. Ann. Bot. 100: 1199-1208.

Welch N (1989). Strawberry production in California. Univ. of California Coop. Ext. Lflt. 2959. ANR Publications, Oakland, CA, USA, p. 15.

Xia G, Cheng L (2004). Foliar urea application in the fall affects both nitrogen and carbon storage in young 'Concord' grapevines grown under a wide range of nitrogen supply. J. Amer. Soc. Hort. Sci. 129: 653-659.