

# Increases in leaf nitrogen concentration and leaf area did not enhance spur survival and return bloom in almonds (*Prunus dulcis* [Mill.] DA Webb)

Sebastian Saa<sup>1</sup> · Eduardo Fernández<sup>1</sup> · Saiful Muhammad<sup>2</sup> · Andres Olivos-Del Río<sup>2</sup> · Theodore M. DeJong<sup>2</sup> · Emilio Laca<sup>2</sup> · Patrick Brown<sup>2</sup>

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**Abstract** Mature almond trees bear fruit mainly on short shoots called spurs, with only a small percentage of fruit produced laterally on long 1-year-old shoots. As a result, maintenance of large numbers of healthy spurs per tree is critical for fruit production. However, spurs that bear fruit have lower leaf area, leaf nitrogen content, and CO<sub>2</sub> assimilation rate than non-fruiting spurs. This has been correlated with reduced percentages of spur survival and return bloom the following season. Thus, we hypothesized that spur leaf area, and ultimately spur health could be enhanced through application of foliar sprays and soil nitrogen treatments that would enhance leaf nitrogen content and spur leaf area. To test our hypothesis, we selected almond trees exhibiting significant yield differences as a consequence of differential soil rates of nitrogen fertilization (N rate) for three prior years (140, 224, and 392 kg/ha). In each tree, three spur types [non-fruiting spurs (F0); spurs with one fruit (F1); spurs with two fruit (F2)] were selected on the east side of the canopy and tracked for one complete season (2011–2012). Four foliar treatments (nutrient replacement, nutrient replacement with biostimulant, nitrogen, and non-spray) were directly applied to individual spurs in each N rate in the spring of 2011 and characteristic such as leaf nitrogen, and fruit quality were recorded

throughout the season. In winter of 2012, spur survival and return bloom were addressed through individual visual inspection of the tagged spurs. In this experiment, soil and foliar N treatments effectively increased spur leaf area, fruit, and leaf nitrogen concentration. In the high N treatment, the leaf nitrogen values exceeded the critical nitrogen concentration established for almond trees and the critical leaf area for spur survival and blooming thresholds established by past research in this area. However, none of these positive changes in leaf N or leaf area improved spur survival and/or return boom of any spur type. Indeed, survival and hull + shell weight tended to be lower in the population of fruiting spurs with the highest leaf area and leaf nitrogen concentration and the return bloom probabilities were always lower in fruiting spurs than in non-fruiting spurs, independent of the nitrogen rate. These results and the relationship between nitrogen rate and spur survival are discussed.

**Keywords** Spur behavior · Leaf area · Nitrogen · Almonds flowering · Foliar sprays

## Introduction

In mature almond trees, spurs (compact shoot structures less than 5 cm long) serve as the fundamental bearing units, with only a small percentage of fruit produced laterally on long 1-year-old shoots. The number of viable spurs in a given year and the survival of spurs between years is thus a fundamental determinant of tree yield. Spur development and the phenology of leaf, fruit, and bud formation on spurs have been well described (Egea et al.

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✉ Sebastian Saa  
sebastian.saa@pucv.cl

<sup>1</sup> Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso, Casilla 4D, Quillota, Chile

<sup>2</sup> Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA

2009; Hawker and Buttrose 1980; Kester et al. 1996; Weinbaum and Muraoka 1986).

Fruit growth (increases in fruit dimensions) begins with fertilization of the flower and reaches 90% of its final size after approximately 4 weeks (Egea et al. 2009). Spur leaf growth and root growth occur simultaneously with the early stages of fruit growth (Kester et al. 1996). Fruit growth consists of a short period of cell division (up to about 2 weeks after pollination), which is followed by a period of cell expansion (Hawker and Buttrose 1980; Kester et al. 1996; Weinbaum and Muraoka 1986), that together represents approximately 60% of the total annual fruit N demand (Muhammad et al. 2015). Fruit growth is significantly affected by the temperature during the first 90 days after flowering (Tombesi et al. 2010). Once the fruit reaches its maximum dimensions and spur leaf growth is complete, shell hardening and kernel fill (embryo growth) take place, which is also characterized by a high nitrogen demand (Nortes et al. 2009). In late summer, flower differentiation for the next season takes place. This transition is not uniform and spurs with higher leaf area are likely to have a greater number of floral buds, and to commence differentiation earlier than buds in spurs with lower leaf area (Kester et al. 1996; Polito et al. 2002).

It is currently hypothesized that individual spurs in an almond tree behave as semi-autonomous structures with respect to their carbon gain (at least in the second part of the growing season), their survival, and their return bloom (Heerema et al. 2008; Lampinen et al. 2011; Saa and Brown 2014; Tombesi et al. 2011). Late season and winter spur survival and return bloom are thought to be correlated with the ability of the leaves on a particular spur to support the spur's carbon demand, independent of the tree as a whole. This concept of semi-autonomy was apparent when Reidel et al. (2004) noted that spurs that bore fruit in the previous season are 80% more likely to die prior to the following spring than non-bearing spurs. These results were further validated by Heerema et al. (2008), Lampinen et al. (2011), and Tombesi et al. (2015). Both Heerema et al. (2008), and Lampinen et al. (2011) also demonstrated that the presence of fruit was associated with reduced spur leaf area and that spurs with higher leaf area have higher survival and return bloom probabilities.

Lampinen et al. (2011) concluded that fruiting spurs with a leaf area  $>28 \text{ cm}^2$  could accumulate sufficient carbohydrates to not only support current fruit load, but also to allow spur survival for the following season, while the survival of fruiting spurs with a leaf area  $<28 \text{ cm}^2$  was significantly reduced. In the same study, Lampinen et al. (2011) also observed that on average, a leaf area  $>45 \text{ cm}^2$  was associated with a significantly higher flowering probability for non-fruiting spurs. Spur mortality is correlated with lower leaf nitrogen content (Saa and Brown 2014) and

reduced leaf area on spurs with fruit (Heerema et al. 2009; Lampinen et al. 2011; Polito et al. 2002; Reidel et al. 2004). Saa and Brown (2014), concluded that the fruit competes for nitrogen with its surrounded leaves in the same spurs. The authors observed that this competition had a significantly negatively effect on the carbon assimilation rate of fruiting spurs while compared to non-fruiting spurs.

The role of nitrogen on leaf area and carbon assimilation rate has not only been documented in almonds (Bi et al. 2003; Egea et al. 2009; Nortes et al. 2009; Saa and Brown 2014), but also in a number of species including, peach, nectarine, and apples (Bi et al. 2003; DeJong et al. 1989; Evans 1989; Rosati and DeJong 2003; Rosati et al. 1999; Xia et al. 2009). Research has been conducted in spur bearing habit trees different from almonds, such as apples and cherries. In apples, Xia et al. (2009) found that higher nitrogen rates increased total leaf area per tree and also enhanced single leaf and whole canopy  $\text{CO}_2$  assimilation. Similarly in cherries, higher leaf area per fruit increased fruit size, and nitrogen availability played a critical role in determining leaf area per fruit ratio (Ouzounis and Lang 2011). Moreover, Ouzounis and Lang (2011) showed that increasing N content through foliar urea resulted in a significant increase in spur leaf area of approx. 25% in cherry trees.

To better understand the role of nitrogen and leaf area on fruit production, survival, and return bloom of fruiting and non-fruiting spurs, we applied targeted applications of nutrients to spur leaves of trees grown under three levels of soil N supply. We hypothesized that foliar and soil fertilization will positively affect leaf-nutrient content and spur leaf area resulting in higher carbohydrate production, which will result in higher probabilities of spur survival and return bloom.

## Methods

### Trees and spurs selection

The experiment was conducted in a drip irrigated commercially managed and highly productive almond orchard, planted in 1998, located near Belridge (35°N; 119°W) CA. Trees were planted at 7.3 m between and 6.4 m within rows, alternating every other row with the variety "Nonpareil" with the pollinizer "Monterrey" (50%). Total water applied during the growing season was equal to 1473 mm with an additional 261 mm occurring as rain almost exclusively in the dormant period. One hundred and forty-four commercial almond trees variety "Nonpareil" exhibiting significant differences in yield and tree nitrogen status as a consequence of differential rates of nitrogen fertilization for three prior years (140, 224, and 392 kg/ha)

were selected (48 trees per nitrogen rate). Yield and leaf N analysis collected in 2010 suggested that these fertilizer rates provided deficient, just adequate, and excessive N supply, respectively. More detailed information regarding to the previous nitrogen management and tree nutrient status can be found in Muhammad et al. (2015), since this experiment is part from a long-term, multidisciplinary, research project. In each tree, eleven spurs per category [non-fruiting spurs (F0); spurs with one fruit (F1); spurs with two fruit (F2)] were carefully chosen for uniform light exposure at the east most outer side of the canopy and tracked for one complete season (2011–2012). Four foliar treatments were randomized at the tree level and applied three times during the spring of 2011.

### Spur sampling

Spur leaf area was destructively sampled and recorded on a sub-population of 144 tagged spurs, using a portable scanner (CanoScan LIDE110) and an image analysis program (ImageJ, US National Institutes of Health, Bethesda, MD, USA). Leaves from the sampled spurs were dried at 65 °C in an oven for a period of 3 days. Then, samples were weighed using a laboratory scale (Sartorius CP324S), and hand ground with a mortar prior to submission to the UC Davis Stable Isotope Facility for their total leaf nitrogen concentration through the combustion method (AOAC 2006).

Fruit drop of the tagged spurs was recorded after the last natural drop characteristic of almond trees (known as “June Drop”). In the summer, labeled spurs that showed symptoms of hull rot infection (*Monilinia fructicola* or *Rhizopus stolonifer*) were eliminated from the study (approx. 16% of the tagged spurs) to avoid confounding effects with the survival data. A greater number of F2 spurs were infected with hull rot under the high N treatment than under the low and medium N treatments, and therefore, data are presented as a percentage of the remaining population to allow comparison between treatments. Finally, spur survival and spur bearing status were assessed during the winter of 2011–2012. Spurs that had developed flower buds were recorded as surviving spurs with return bloom. Spurs that developed vegetative buds, but not flower buds, were recorded as surviving spurs only. Spurs that had neither vegetative nor floral buds were recorded as death spurs.

### Foliar treatments

Preliminary experiments in trees at this same location were conducted in 2009 to determine the effect of fruiting status on spur leaf-nutrient concentrations. This preliminary experiment demonstrated that the presence of fruit on a

spur resulted in a statistically and significant reduction in leaf tissue concentrations of N, P, K, S, Zn, and Cu, when compared with non-fruiting spurs (Table 1). Three spray applications were applied at weekly intervals commencing 28 days after full bloom (DAFB) and covering the period of exponential fruit growth and leaf growth. Foliar treatments are described in Table 2 and included: control (no spray); foliar N (N); depleted nutrient replacement (NR); and a fourth treatment consisting of nutrient replacement plus a biostimulant treatment that has been shown to be effective in preliminary trials (NR + B). Sprays were applied using hand sprayers targeting individual spurs. 1584 spurs in each spur category (F0/F1/F2) on 144 trees were sprayed for a total of 4752 individual spurs. The application of the NR + B treatment resulted in spur toxicity and lower survival probabilities than the unsprayed control, and therefore, it was eliminated from the analysis. Thus, results from NR + B will not be included and neither further discussed.

While the number of spurs sprayed in this experiment was large, the percent of spurs on any single tree that received any kind of foliar treatment was very low representing less than 0.005% of the total number of fruiting spurs on the tree. Foliar sprays, therefore, should not have altered whole-tree yield, whole-tree leaf area, or any other whole-tree characteristic.

### Leaf and fruit nutrient analysis

Non-fruiting spur leaves (F0), fruiting spur leaves (F1, F2), and fruit (from F2 spurs) from non-tagged spurs were collected 128 DAFB from trees in each soil N treatment plot that did not receive foliar sprays (from the control-foliar-trees). Leaf samples were ground using a Wiley mill to pass a 40-mesh screen and sent to the UC Davis analytical lab for the analysis of nitrogen through the combustion method (AOAC 2006). Fruit samples were weighed and total kernel weight and hull + shell weight was recorded (total fruit weight = kernel weight + hull + shell weight) prior to being sent to the lab for N analysis. Similarly, fruit from tagged spurs was individually collected at full harvest maturity (180 DAFB) and processed as described above.

### Experimental design, statistical analysis, and model development

The experimental design was a split-split plot with a  $3 \times 4 \times 3$  factorial structure, in which main plots were the variable soil nitrogen rates, sub-plots were the foliar treatments, and the sub-sub-plots were the different types of spurs. Four blocks were established to reduce spatial variability in the orchard from south to north. One block

**Table 1** Preliminary experiment showing nutrient differences in leaves from almond spurs

Sample type	<i>n</i>	N (%)	P (%)	K (%)	S (%)	Zn (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )
NF	30	2.40a	0.12a	1.42a	0.15a	37a	4.9a
F1	30	2.23b	0.10b	1.37b	0.13b	34b	4.7b
F2	30	2.11c	0.10b	1.15c	0.13b	31c	4.5b

Leaves from spurs containing no fruit (NF), single fruit (F1) and two fruits (F2) were sampled from 30 commercial almond trees in late July 2010 and analyzed for 11 essential elements. Macronutrients are presented in % of dry weight and micronutrients are presented in mg kg<sup>-1</sup> of dry weight. Different letters symbolize significant differences accordingly to Tukey *t* test with an alpha level = 0.05

**Table 2** Composition of foliar sprays

Treatment	Formulation	Element	Concentration of nutrient in final spray solution (mg kg <sup>-1</sup> )
Foliar N (N)	Nitrogen (urea-Triazone)	N	3592
Targeted nutrient replacement plus biostimulant (NR + B)	Multi-element mix plus biostimulant	N, P, K, S, Co, Cu, Fe, Mn, Zn, B Grozyme	6964, 7218, 2168, 88, 2.2, 22.5, 44.5, 44.2, 90.6, 0.1
Targeted nutrient replacement (NR)	Multi-element mix	N, P, K, S, Zn, Cu	1800, 1800, 3960, 154, 228, 22
Control	No-application		0

had all the soil fertilization treatments (main plots), which were a group of three rows with 15 trees per row each. In the middle row of each main plot, two guard trees were left at each side and all the foliar treatments (sub-plots) were randomized among the other 12 trees. The first and third rows of each main plot served as buffer rows. Each sub-plot was a set of three trees and a tree between each foliar treatment was left as guard. Finally, in each sub-plot, three different spur types of categories of eleven spurs each were selected at the three level (sub-sub-plot).

Parametric data such as spur leaf area, leaf nitrogen concentration, and leaf and fruit weight were statistically analyzed using the JMP program version 12, SAS Institute Inc., Cary, NC, USA, 1989–2010.

Both survival and return bloom were modeled using generalized linear mixed models with the lme4 package (Bates et al. 2013) in R Core Team (2012). The models for survival and return bloom written in R syntax were:  $\text{glmer}(\text{cbind}(\text{alive}, \text{dead}) \sim \text{Nrate} * \text{foliar} * \text{spurtrt} + (1|\text{rep}) + (1|\text{main}) + (1|\text{treeID}), \text{family} = \text{binomial}, \text{data} = \text{spurs})$ , and  $\text{glmer}(\text{cbind}(\text{flor}, \text{veg}) \sim \text{Nrate} * \text{foliar} * \text{spurtrt} + (1|\text{rep}) + (\text{Nratelmain}) + (1|\text{treeID}), \text{family} = \text{binomial}, \text{data} = \text{spurs})$ . These models were obtained by simplifying the full set of random effects according to the procedures described in Pinheiro and Bates (2000), essentially by keeping all random effects that were statistically significant. Assumptions about the random effects and within-group residuals were checked following the graphical procedures described in section 4.3 of Pinheiro and Bates (2000). Significance of parameter estimates was checked in the `summary()` function of the models and expressed as tables of Wald's tests obtained with the `Anova()` function of

the car package (Fox and Weisberg 2011). Treatment differences were assessed by inspection of the table of effects and their standard errors. Finally, all selected outputs were plotted using Sigma Plot program version 12.0, Systat Software, Inc., San Jose, CA 95110, USA.

## Results

### Nitrogen status

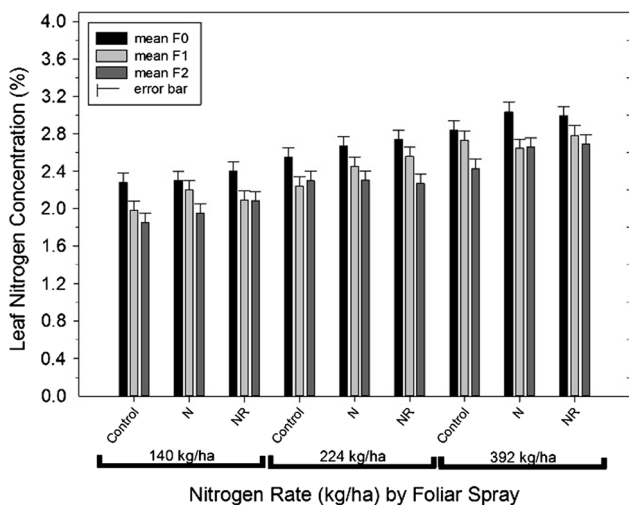
Soil N rate ( $p < 0.01$ ), and spur type ( $p < 0.01$ ) significantly affected leaf nitrogen concentration at 91 DAFB, and no significant interaction was detected among these factors. Applications of 140, 224, and 392 N kg/ha resulted in significantly different leaf N concentration (by dry weight) values of 2.1, 2.5, and 2.8%, respectively. Higher soil N applications significantly increased leaf nitrogen concentration in all spur categories and fruiting spurs had significantly lower nitrogen concentrations than non-fruiting spurs (though this difference tended to be less under the high nitrogen rate treatment). F0-no-sprayed-spurs receiving low soil N applications (140 kg N) averaged 2.27% N, while two fruiting spurs averaged 1.8% N. F0-no-sprayed-spurs receiving high soil nitrogen application rates (392 kg N), averaged 2.8% N, while two fruiting spurs averaged 2.4% N. Foliar sprays had a positive effect over the control spray, being foliar NR more effective than foliar N. The biggest effect of NR sprays was detected in F2 spurs at 140 and 392 kg N, while N spray only had a positive effect on 392 kg N (Fig. 1).

For non-sprayed spurs in trees receiving high soil nitrogen application rates at 128 DAFB, leaf nitrogen values were significantly above the critical concentrations [N = 2.2%, Reuter and Robinson (1997)] in all spur categories. On the other hand, leaves on bearing spurs of trees grown at intermediate or low soil N applications had marginal or deficient nitrogen concentration (Fig. 2).

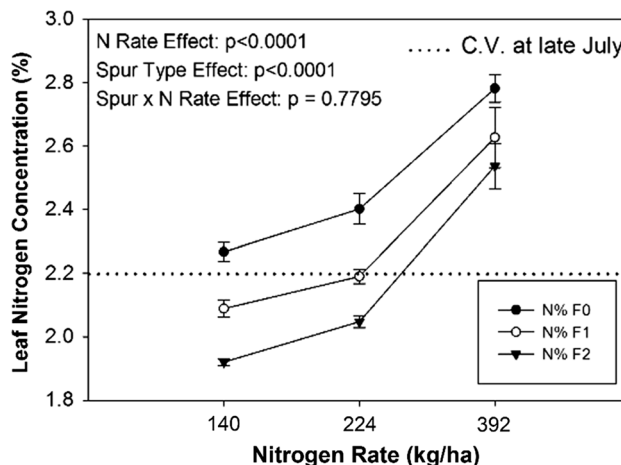
For nitrogen in the fruit, only the results at 180 DAFB are presented, because the same trends were observed at the 128 DAFB and 180 DAFB fruit sampling times. Kernel nitrogen concentration was only significantly affected by soil N rate ( $p < 0.01$ ), while hull + shell N concentration was significantly affected by soil N rate and spur type. N concentration of kernel and hull + shell was higher for trees receiving the high nitrogen treatment than those receiving the low or intermediate nitrogen treatments. Kernel N concentration increased significantly from 3.25% in the low nitrogen rate treatment to 4% in the high nitrogen treatment. Hull + shell N values increased from 0.6% in the low and intermediate soil N treatments to 1.2% in the high N treatment. Hull + shell N concentration was also consistently higher in fruit from F1 spurs than from F2 spurs irrespective of the nitrogen rate (Fig. 3a, b).

**Spur leaf area, fruit drop, and fruit weight at harvest**

Spur leaf area recorded at 91 DAFB was significantly affected by soil nitrogen application rate ( $p < 0.01$ ) and the type of spur ( $p < 0.01$ ). Foliar sprays ( $p = 0.09$ ) and their



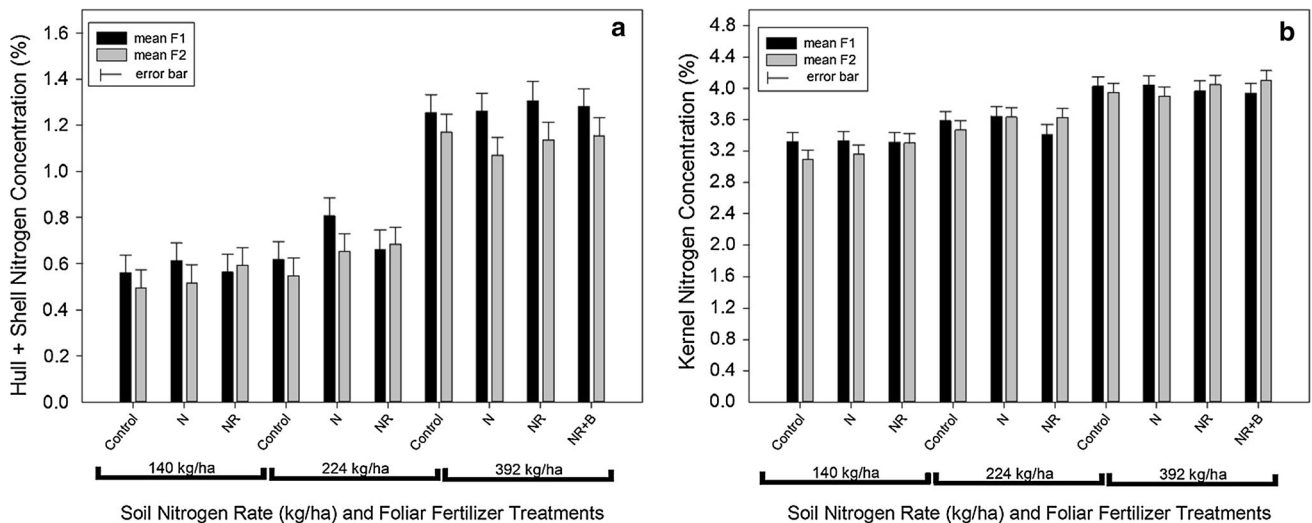
**Fig. 1** Effects of soil nitrogen rate (140, 224, and 392 kg/ha), foliar treatment (control, N, NR), and spur type (F0, F1, F2) on leaf nitrogen concentration (%) recorded at 91 DAFB ( $n = 144$  spurs). Bars indicate standard error from the mean



**Fig. 2** Leaf nitrogen concentration (%) against soil nitrogen rate (140, 224, and 392 kg/ha) and spur type (F0, F1, and F2). Data were collected at 128 DAFB using non-sprayed trees (foliar control trees). Dashed line represents the standard critical value concentration (C.V.) for nitrogen in almond trees ( $n = 36$  pooled samples). Bars indicate standard error from the mean

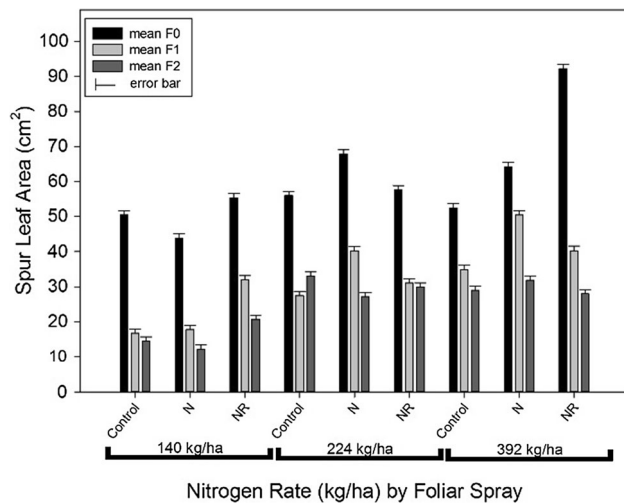
interaction with the soil nitrogen applications ( $p = 0.08$ ) had a non-significant effect on spur leaf area. Spur leaf area in non-sprayed-F2-spurs went from 12.8 cm<sup>2</sup> for the 140 kg N treatment to 27.4 cm<sup>2</sup> for the 392 kg N treatment. On the other hand, spur leaf area in non-sprayed-F0-spurs had very little variation across the different nitrogen rates, constantly averaging 51 cm<sup>2</sup>. NR effectively increased leaf area for the 140 kg N treatment in all spur types. F0, F1, and F2 spurs from trees of 140 kg N treatment increased their leaf area by 10, 100, and 58%, respectively, when they were sprayed with NR. Similarly, NR sprays increased spur leaf area by 75 and 15% in F0 and F1 spurs from trees receiving 392 kg N rate. N sprays also had a beneficial effect on spur leaf area. Spurs that received N sprays had higher leaf area than their controls by 22 and 46% on F0 and F1 spurs, respectively, in the 392 kg N rate (Fig. 4).

Hull + shell weight at 128 DAFB was significantly reduced from 1.75 to 1.47 g with increasing nitrogen rate across, and at 180 DAFB, hull + shell weight was reduced from 2 to 1.6 g with increasing nitrogen rate across both spur types. Kernel weights of 0.65 and 1.12 g were recorded at 128 DAFB and 180 DAFB, respectively, and were not significantly affected by N application rate or spur type (Table 3). The reduction of shell + hull weight as the N rate increased was partially (not significantly) mitigated by the applications of N and NR sprays. In agreement with observations over the preceding three seasons, 392 kg of soil N per ha significantly increased tree yield during the 2011 season (Table 4). Fruit drop on F1 and F2 spurs averaged 22% and was not affected by any treatment.



**Fig. 3** Effects of soil nitrogen rate (140, 224, and 392 kg/ha), foliar treatment (control, N, and NR), and spur type (F0, F1, and F2) on Hull + Shell nitrogen concentration at harvest (180 DAFB) (a), and on

kernel nitrogen concentration at harvest (b). Bars indicate standard error from the mean



**Fig. 4** Effects of soil nitrogen rate (140, 224, and 392 kg/ha), foliar treatment (control, N, and NR), and spur type (F0, F1, and F2) on spur leaf area recorded at 91 DAFB. Bars indicate standard error from the mean

### Spur survival and return bloom

Spur survival probabilities ranged from 74 to 12%, being significantly affected by the spur type ( $p < 0.01$ ) and nitrogen rate ( $p < 0.01$ ). Fruiting spurs had significantly lower survival rates than non-fruited spurs. F2 spurs had survival rates that averaged 15%, while F1 spurs and F0 spurs had survival rates of 39 and 64%, respectively, across all treatments. The survival rates of all spurs were significantly decreased under the high nitrogen application rates when compared to medium and low soil N rates with overall survival values of 35% under high N and 45% under low N. No significant effect of the foliar treatments

on spur survival was observed ( $p = 0.1$ ), with only marginal benefit from the N spray treatment (Fig. 5a).

Return bloom probabilities, which were conditional upon the survival of the tagged spurs, were only significantly affected by spur type. No significant effect of nitrogen rate or foliar spray was detected. Fruiting spurs had significantly lower bloom probabilities than non-fruited spurs. F2 spurs had bloom probabilities that averaged 28%, while F1 spurs and F0 spurs had bloom probabilities of 45 and 78%, respectively, across all treatments (Fig. 5b).

### Discussion

Preliminary experiments in trees at this same location were conducted in 2009 to determine the effect of fruiting status on spur leaf-nutrient concentrations. This preliminary experiment demonstrated that the presence of fruit on a spur resulted in a statistically and significant reduction in leaf tissue concentrations of N, P, K, S, Zn, and Cu, when compared with non-fruited spurs (Table 1). In addition, research from Heerema et al. (2009) and Saa and Brown (2014) points out that nitrogen is a key element for spur survival.

Prior research (Heerema et al. 2008; Lampinen et al. 2011; Polito et al. 2002; Saa and Brown 2014; Tombesi et al. 2011; Tombesi et al. 2015) suggests that spur survival and return bloom are largely determined by bearing status, leaf nitrogen content, and leaf area of the individual spurs, and that these spur level effects are a consequence of competition for carbohydrates and nutrients between fruit and spur. To further test this hypothesis, we independently

**Table 3** Hull + shell weight (g) and kernel weight (g) sampled at 128 days after full bloom (DAFB) and 180 DAFB

Nitrogen rate (kg/ha)	Spur type	Hull + shell weight (g)		Kernel weight (g)	
		128 DAFB	180 DAFB	128 DAFB	180 DAFB
140	F1	NA	2.04a	NA	1.14a
140	F2	1.75a	1.84**a	0.68a	1.09a
224	F1	NA	1.77b	NA	1.11a
224	F2	1.58b	1.69**b	0.65a	1.12a
392	F1	NA	1.7c	NA	1.13a
392	F2	1.47c	1.64**c	0.63a	1.11a

At 128 DAFB, only two fruiting spurs (F2) were sampled, while at 180 DAFB, both spur types were sampled (F1). Different letters in the same column represent significant differences between different nitrogen rates. Asterisks in the same column represent significant differences between different spur types under the same nitrogen rate

**Table 4** 2011 Kernel yield expressed in kg/ha

N rate (kg/ha)	Least square mean (kernel kg/ha)
392	4478a
224	3577b
140	2942c

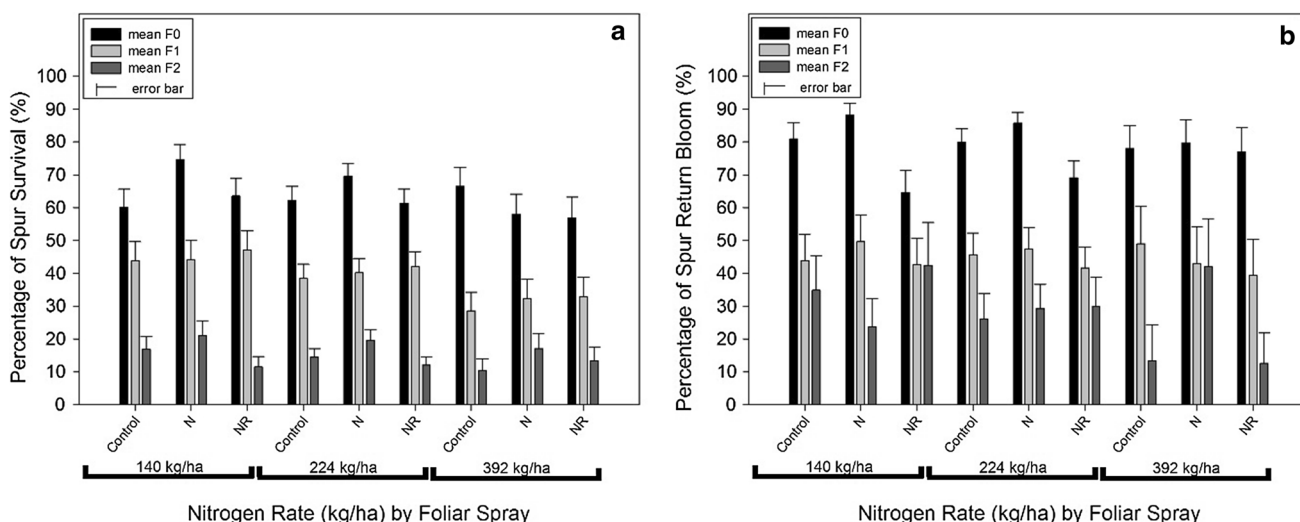
The yield of each experimental unit (group of 12 trees per repetition/treatment combination) was individually recorded at 180 DAFB. Different letters in the column “least square mean” represent significant differences between different nitrogen rates

manipulated whole-tree and spur N levels and directly altered spur source-sink relationships, and examined the effects of these manipulations on spur survival and return bloom.

Increments in leaf area and/or leaf nitrogen content of an individual spur or shoot have been shown to increase carbon assimilation under varied light status (DeJong et al.

1989; Rosati and Dejong 2003; Rosati et al. 1999; Xia et al. 2009), and this has been associated with increased spur survival and return bloom (Heerema et al. 2008; Lampinen et al. 2011). Increments in N concentration up to a maximum corresponding to the leaf critical nutrient concentration also enhance carbon assimilation rate by directly affecting the enzymes of the Calvin cycle (Chen and Cheng 2004; Prsa et al. 2007; Saa and Brown 2014; Yamori et al. 2011). Increases in spur leaf area would be expected to enhance carbon assimilation per spur by enhancing the amount of light intercepted per spur. Thus, spurs with higher leaf nitrogen and higher leaf area have the potential to produce larger fruit and/or fruit with higher N content while simultaneously increasing the amount of carbon storage and nutrient available for spur survival and/or return bloom.

In this experiment, soil and foliar N treatments effectively increased spur leaf area, fruit, and leaf nitrogen concentration. Treatment imposed changes in leaf N were



**Fig. 5** Effects of nitrogen rate (140, 224, and 392 kg/ha), foliar treatment (control, N, and NR), and spur type (F0, F1, and F2) on the survival (a, n = 2660) and return bloom (b, n = 1056) of the tagged spurs. Bars indicate standard error from the mean

statistically significant. Thus, with the high soil nitrogen treatment, leaf-nutrient concentrations of all spur types, including bearing spurs, were increased to above the established critical value of 2.2%, while leaves of all the spur categories in the lowest soil nitrogen rates were at or below the established critical value. Spur leaf area increased as applied soil N increased in all F0 spurs and in F1 spurs of trees supplied with 224 or 392 kg/ha of N, and in F2 spurs of trees provided with 392 kg/ha N, thus exceeding the spur survival and flowering thresholds of 28 and 45 cm<sup>2</sup> established by Heerema et al. (2008) and Lampinen et al. (2011). Given the observation that leaf area increased with soil and foliar N applications and on the basis of prior results (Lampinen et al. 2011; Saa and Brown 2014), it can be assumed that treatments used here meaningfully increased net spur carbon assimilation and hence would be expected to decrease the magnitude of carbon deficit that occurs in fruiting spurs and in non-fruiting spurs, if any. Likewise, the strong positive effects of soil and foliar N treatments on leaf and fruit N indicate that N competition between fruit and spur was at least partially mitigated and hence an increase in spur survival and return bloom would have been expected.

In agreement with past research, spur survival and bloom capacity were negatively affected by the spur bearing status in the current season with F0 spurs exhibiting survival and bloom rates much higher than F1 and F2 spurs. The previous research has hypothesized that these effects of fruiting on spur survival and return bloom are a consequence of competition between fruit and spurs for carbohydrates and nutrients. In apparent contradiction to this theory, neither the increment in leaf nitrogen concentration due to the nitrogen rates nor the increment in leaf area as a consequence of foliar treatments seen in this experiment improved survival rates in any spur type and/or modified the return bloom of any spur type as would be predicted by the model developed by Lampinen et al. (2011). Indeed, survival tended to be lower in the population of fruiting spurs with highest leaf area and leaf nitrogen concentration and the return bloom probabilities were always lower in fruiting spurs than in non-fruiting spurs, independent of the nitrogen rate.

The observed negative effect of increased soil N on spur survival, and hull + shell weight may be a consequence of the significant increase in whole-tree yield as soil N increased, thus suggesting that spurs are less autonomous than previously suggested (Heerema et al. 2008; Lampinen et al. 2011; Tombesi et al. 2011). In the current experiment, overall, spur survival of F0 spurs in 2012 was lower than previously observed by past research (Heerema et al. 2008; Reidel et al. 2004), though survival of F1 spurs was approximately equivalent (Reidel et al. 2004), and there is no past research on F2 spurs. The lower spur survival

observed here in F0 spurs would reduce the potential for observing treatment effects.

Alternatively, the fact that spur behavior was negatively affected by high nitrogen rates could be attributed to differences in light interception among trees. As nitrogen fertilization increased, yield increased, and so did the size of the tree (Muhammad et al. 2015). Therefore, spurs from high nitrogen rates are more likely to be under shaded positions than spurs under lower nitrogen rate. However, this potential issue can be discarded in this experiment, since the spurs selected here were always selected from the outside part of the canopy. Moreover, specific leaf area, a measure well correlated with light interception (Dejong and Doyle 1985; Heerema et al. 2008; Klein et al. 1991; Lampinen et al. 2011), was not significantly different between nitrogen treatments (data not shown).

We hypothesized in agreement with Heerema et al. (2009) and Saa and Brown (2014) that fruit induced competition for N may also reduce spur survival. This possibility can also be excluded, since leaf, hull + shell, and kernel N were all higher in trees provided with increasing soil N, suggesting that N limitation did not occur in high N trees at any stage of crop phenology. High tree yield may also have acted to reduce spur survival through effects on whole-tree carbon balance. However, this would be a contradiction to the semi-autonomous theory of Heerema et al. (2008) and others (Lampinen et al. 2011; Tombesi et al. 2011). The significantly higher leaf area measured at 91 DAFB of the high yielding-high soil N rate treatments suggests that any yield induced reduction in whole-tree carbon status was not apparent at 91 DAFB. In contrast, the significantly reduced hull + shell weight detected at 128 DAFB and even stronger at harvest (180 DAFB) of the higher yielding-high soil N trees indicates that there may have been a competition for carbon to satisfy the larger crop with a concurrent reduction in carbon available to each spur. Thus, high soil N in these experiments likely increased whole-tree yield through increased tree size and fruiting positions (Muhammad et al. 2015), but decreased spur level survival, perhaps, by inducing a carbon resource deficit, thereby reducing per fruit hull + shell weight and reducing spur survival. During the early season, spurs appeared to behave as largely autonomous units, while later in the season (>91 DAFB), spur behavior may have been more influenced by whole-tree crop load. The possibility that high tree N status altered the exposure of experimental spurs to light, and thereby reduced carbon assimilation, at later stages of the experiment, cannot be excluded. However, the amount of light between spur types and between foliar treatments under the same nitrogen rate must have been very similar through the whole season. These findings do not fully support the semi-autonomous theory of Heerema et al. (2008) and Lampinen



et al. (2011), in which they state that spurs are dependent on whole-tree carbon early in the season and more autonomous later in the season.

In a recent review of the effects of fruiting on return bloom in almond, Tombesi et al. (2011) suggested that there are at least two non-exclusive hypotheses that may explain the role of spur bearing status on productivity of those spurs in the subsequent season. The first hypothesis suggests that the production of hormones by the fruit and seeds can act to reduce floral bud viability, and the second hypothesis suggests that high sink demand decreases leaf function by negatively affecting the early delivery of nutrient and/or carbohydrates to the leaf on fruit bearing spurs.

Plant growth regulators have been shown to have a negative effect on return bloom in many crops (Gonzalez-Rossia et al. 2007; Martinez-Fuentes et al. 2010; Reig et al. 2011; Reig et al. 2006). Hormones, such as gibberellins, are naturally produced by the endosperm of growing seeds (Schmidt et al. 2009) and their flower inhibition effect is apparent when flower differentiation overlaps with seed formation (Martinez-Fuentes et al. 2010). Martinez-Fuentes et al. (2010) showed that the negative effect of citrus fruit on return bloom was only evident once fruit had completed 90% of its final size. In addition, the authors concluded that this inhibitory effect was independent of the leaf-carbohydrate-contents once a minimum carbohydrate threshold (as energy source) was fulfilled. Similarly, Smith et al. (2007) showed that neither carbohydrates nor nitrogen is the limiting factor for return bloom in well-managed pecan trees.

In almond trees var. Nonpareil, flower bud initiation occurs approx. 150–180 DAFB (Lamp et al. 2001), coinciding with the presence of recently developed mature seeds (kernels) that could inhibit return bloom through hormone production or through negatively affecting nutrient and/or carbohydrate demand (Tombesi et al. 2011). Our experiment successfully manipulated spur nutrient and potentially carbohydrate supply through increments in leaf area, leaf nitrogen content, and total tree yield. However, none of these changes had an effect on the return bloom probabilities of the different spur types. Non-fruiting spurs always had higher return bloom probabilities than F1 and F2 spurs, and these probabilities were constant across all treatments. Thus, in view of the results of this study and in agreement with the results obtained in other species (Chan and Cain 1967; Gonzalez-Rossia et al. 2007; Martinez-Fuentes et al. 2010; Neilsen and Dennis 2000; Reig et al. 2011; Schmidt et al. 2009; Smith et al. 2007), we hypothesize that the return bloom of almond spurs is more restricted by factors such as hormones rather than the ability of the spur's leaves to supply sufficient carbohydrates or nutrients to support return bloom.

**Author contribution statement** SS designed the experiment and collected the data with SM and AO. SS and EF wrote the manuscript. TD and EL improved the manuscript and aid with statistical analysis. PB supervised the research and guided SS during this process.

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