Title: Spur Sub-Populations, Fruiting and Leaf Senescence as a Possible Refinement of Diagnostic Leaf Sampling to Determine Tree N Status

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Introduction

Productivity in almond is very closely tied to soil nitrogen (N) availability and, in order to apply N optimally and efficiently in the orchard, growers need a sensitive diagnostic indicator of tree N status. Currently, the standard gauge of tree N status in the California almond industry is the N concentration of leaves sampled randomly from <u>non-fruiting spurs</u> in mid-July. Our understanding of spur biology and canopy nitrogen allocation has improved in recent years, but the recommended leaf-sampling protocol has remained unchanged for many decades. Spurs may function as independent (or "autonomous") sub-units such that, within a tree canopy, N stresses are confined mainly to certain vulnerable spur sub-populations, such as the fruiting spurs. This scenario of localized N stress, if true, could have diagnostic relevance.

We reasoned that a leaf sampling protocol taking advantage of localized spur stress within particular spur sub-populations, specifically the shaded, fruiting spurs (rather than leaf sampling from random, non-fruiting spurs), could potentially serve as a more sensitive nitrogen diagnostic tool. Our rationale was as follows: when root N uptake from the soil is inadequate to meet fruit N demands, the leaves of specifically the fruiting spurs may begin to senesce (die) during the season, while N stress is not yet evident in the leaves of non-fruiting spurs. The leaf senescence process is characterized by a sharp (often about 50%) drop in leaf N due to remobilization of N held by leaf proteins and translocation of this N out of the leaf. A leaf sampling protocol yielding larger differences in leaf N concentration for a given difference in tree N status could possibly allow growers to more readily detect tree N stress and respond more quickly to the problem.

Objective:

To assess the possibility of modifying the currently-accepted leaf sampling protocol to increase the diagnostic sensitivity of leaf analysis in the determination of tree N status.

Materials and Methods

1. *Characterization of spur sub-populations as defined by spur fruiting status and light exposure with regards to their vulnerability to N-deprivation.*

- A range in tree N status (i.e. 'Low N' and 'High N' trees) was created by varying the fertilizer N application rates.
- Hundreds of fruiting and non-fruiting spurs representing a range of light exposure were tagged.
- Specific leaf weight (SLW; leaf dry weight per unit leaf area, an easily measured and reliable indicator of relative leaf light exposure), leaf number per spur, spur survival rates and flower bud number per spur were measured on tagged spurs between April and the subsequent February.

2. *Assessment of the relative sensitivities of the conventional and proposed leaf sampling protocols.*

- Using trees with a range in N-status, changes in leaf N during kernel growth (a major period of carbohydrate and nitrogen demand in the tree) were examined for four different spur sub-populations, defined by spur fruiting status and light exposure.
- The N concentrations of leaves sampled conventionally were compared with those of leaves sampled from selected vulnerable spur sub-populations to determine whether the vulnerable spurs provide evidence of leaf N loss under circumstances in which the conventional protocol is less sensitive.

Results and Discussion

Confinement of Stress Within Vulnerable Spur Sub-Populations: Spur Survival and Flower Bud Initiation

Spur fruiting status and light exposure affect the fate of almond spurs in the following season. Fruiting spurs were far less likely than non-fruiting spurs to initiate flower buds and survive until spring (Table 1). Spur survival (Table 2) and spur flower bud initiation rates (Table 3) increased dramatically with increasing spur light exposure (as determined by increasing SLW) in the previous season, especially among fruiting spurs. Light exposure influences the ability of a spur's leaves to carry out photosynthesis and may, as a result, be positively associated with spur carbohydrate availability. These data are consistent with the concept of a relative spur autonomy –at least late in the growing season- with respect to carbohydrate and N availability. That is, the carbohydrate and remobilized N available for flowering, fruiting and survival of a spur appear to be largely restricted to that originating from the leaves of that particular spur and carbohydrate and N stress are quite localized within individual spurs. Data from the 'High N' and 'Low N' treatments were combined in Tables 2 and 3 because neither flower bud number nor percent spur survival were significantly influenced by the 2003 nitrogen treatment. We cannot conclude from the results of this experiment that reduced yield due to mild N-stress occurs through decreases in spur floral initiation or spur survival rates. It is therefore likely, in almond, that mild N stress reduces tree yields through decreasing shoot growth and rates of spur renewal, decreasing the size of a tree's fruiting spur population.

Confinement of N-stress Within Vulnerable Spur Sub-Populations: Early Leaf Drop.

N-deficient almond trees often exhibit heavy leaf loss before the normal autumn leaf fall and even before harvest. Early leaf loss was not spread randomly among all spurs; rather, fruiting and shaded spurs exhibited heavy early leaf drop, while nearby non-fruiting and well-exposed spurs experienced very little leaf drop before autumn. This demonstrates that N-stresses are mainly confined to these vulnerable spur sub-populations. Irrespective of tree N treatment, non-fruiting spurs (both shaded and exposed) and exposed, fruiting spurs had very low levels of early leaf drop, losing, on average, less than 30 % of their leaves between May and late September, 2004 (Fig. 1). The shaded, fruiting spurs had, by far, the highest leaf drop rates of the spur sub-populations tested. On 'High N' treatment trees, for instance, the leaf drop rate of the shaded, fruiting spurs was about double that of the spur sub-population with the second highest leaf drop rate, the shaded, non-fruiting spurs (Fig.1). The developing kernel represents a very large N demand for an almond spur, but, interestingly, spur stress may have been accentuated by fruit growth or shading long before kernel development began because shaded and/or fruiting spurs already had fewer leaves by the first measurement date.

There were differences among the various tagged spur sub-populations in their response to the tree N treatments. Tree N treatment did not substantially affect leaf drop rates for the non-fruiting spurs or exposed, fruiting spurs in 2004. In contrast, the shaded, fruiting spurs had a much higher leaf drop rate on 'Low N' than 'High N' trees. Shaded, fruiting spurs on 'Low N' trees lost an average of 75% of their leaves, while that same spur category on 'High N' trees lost 57% of their leaves (Fig. 1). Thus, the increase in leaf drop on 'Low N' trees was limited principally to fruiting spurs in shaded canopy positions. The combination of spur shading and fruit growth considerably increase a spur's vulnerability to the N stress that occurs when tree N demand exceeds N uptake by the roots from the soil.

Relative Sensitivities of Leaf N Sampling Protocols.

Leaves sampled from shaded, fruiting spurs could be a more sensitive indicator of N deficiencies than leaves from non-fruiting spurs if tree N availability (tree N demand relative to N supply) affects the leaf N concentration of shaded, fruiting spurs more than that of non-fruiting spurs. In 2003 we concluded that, in marginally N- deficient trees, leaf N concentrations of fruiting spurs did not provide a more sensitive diagnostic indicator of tree N status than did leaf N concentrations of non-fruiting spurs. Though fruiting spurs did have lower leaf N concentrations, the decline in leaf N concentration of persistent leaves during the kernel development period in 2003 was no greater for fruiting spurs than it was for non-fruiting spurs, and the differences in leaf N concentration between 'High N' and 'Low N' trees were the same for fruiting and non-fruiting spurs (data not shown).

A greater range of tree N status was established in 2004 than in 2003, including trees both above and below the currently-accepted threshold for N-deficiency (leaf N concentration of July sampled non-fruiting spur leaves of 2.2%). In July 2004, the leaf N concentrations of shaded, fruiting spurs on the 'High N' and 'Low N' trees were 2.3% and 1.5%, respectively, while that of the conventionally-sampled leaves (that is, from exposed, non-fruiting spurs) were 2.4% and 1.7%, respectively (Fig. 2). Thus, there existed a large difference in tree N status- the 'Low N' trees were well below the adequate N range, and the 'High N' trees were well within the adequate N range- but, as in 2003, when 'High N' and 'Low N' trees were contrasted, the difference in leaf N concentration was about the same for shaded, fruiting spurs (0.8%) as it was for exposed, non-fruiting spurs (0.7%). As was discussed previously, nitrogen stress (indicated by higher rates of early leaf drop) was accentuated in the shaded, fruiting spurs. We anticipated that there would also be greater early leaf senescence (death) on shaded, fruiting spurs and, consequently, movement of N out of the leaves to the developing fruits on the spur. Compared with non-fruiting spurs, the shaded, fruiting spurs, especially on the 'Low N' trees, began the season with a lower N concentration and had a much smaller percentage decline in leaf N concentration during kernel development. This means that, although the early leaf drop, spur mortality and floral initiation data clearly show that stress appears in shaded, fruiting spurs before non-fruiting spurs, based on the leaf N data, there is no detectible difference in the level of N-stress, in terms of N remobilization, exhibited by the persistent leaves sampled from these two spur types. We conclude that leaves sampled for N analysis from shaded, fruiting spurs do not provide a more sensitive indicator of tree N stress than leaves sampled according to the conventional protocol.

Summary and Conclusions

- Four categories of spurs were studied over several years. These spur categories were:
    1) Well-exposed, non-fruiting spurs.
    2) Shaded, non-fruiting spurs.
    3) Well-exposed, fruiting spurs.
    4) Shaded, fruiting spurs.
- These spur categories experienced different degrees of stress. Indicators of spur stress were the following:
    - Increased early leaf drop (i.e. prior to normal autumn leaf fall).
    - Reduced flower bud formation.
    - Increased spur death over winter.
- The shaded, fruiting spurs were the most stressed spur type, while exposed, non-fruiting spurs were the least stressed spur type.
- Despite clear differences in the stress experienced by the different spur categories, the difference in N concentration between 'High N' and 'Low N' trees was no greater for leaves sampled from shaded, fruiting spurs than for conventionally sampled leaves (i.e. from exposed, non-fruiting spurs).
- N concentration of persistent leaves sampled from shaded, fruiting spurs does not provide a more sensitive indicator of tree N status than does that of conventionally-sampled leaves. Continued use of the current leaf sampling protocol (leaves sampled in July from non-fruiting spurs) is recommended.

Table 1. The relationship of spur fruiting status to subsequent spur survival and return bloom.

Spur Fruiting Status, 2003	Survival, 2004 (%)	Return Bloom, 2004 (%)[z]	Flower Buds per Spur, 2004[y]
Fruiting	86.5	18.9	0.32 ± 0.04
Non-Fruiting	99.2	56.6	1.09 ± 0.06

[z] Percentage of spurs with at least one flower bud, spring 2004.
[y] Data are means ± standard error.

Table 2. The relationship between specific leaf weight (SLW) and survival of single-fruited and non-fruiting spurs.

Spur Fruiting Status, 2003	Spur SLW, 2003 (mg cm-2)[z]	Number of Spurs	Spur Survival, 2004 (%)[y]
Single-Fruited	0-8	121	68.6
	8-10	160	92.5
	10-12	100	98.0
Non-Fruiting	0-8	90	97.8
	8-10	178	99.4
	10-12	100	100

[z] Specific Leaf Weight, leaf dry weight per unit leaf area, is a reliable indicator of relative leaf light exposure.

Table 3. The relationship between specific leaf weight (SLW) and return bloom of single-fruited and non-fruiting spurs.

Spur Fruiting Status, 2003	Spur SLW, 2003 (mg cm-2)[z]	Number of Spurs	Return Bloom, 2004 (%)[y]
Single-Fruited	0-8	83	9.6
	8-10	148	15.5
	10-12	98	29.6
Non-Fruiting	0-8	88	50.0
	8-10	177	55.9
	10-12	100	62.0

[z] Specific Leaf Weight, leaf dry weight per unit leaf area, is a reliable indicator of relative leaf light exposure.
[y] Percentage of spurs with at least one flower bud, spring 2004.

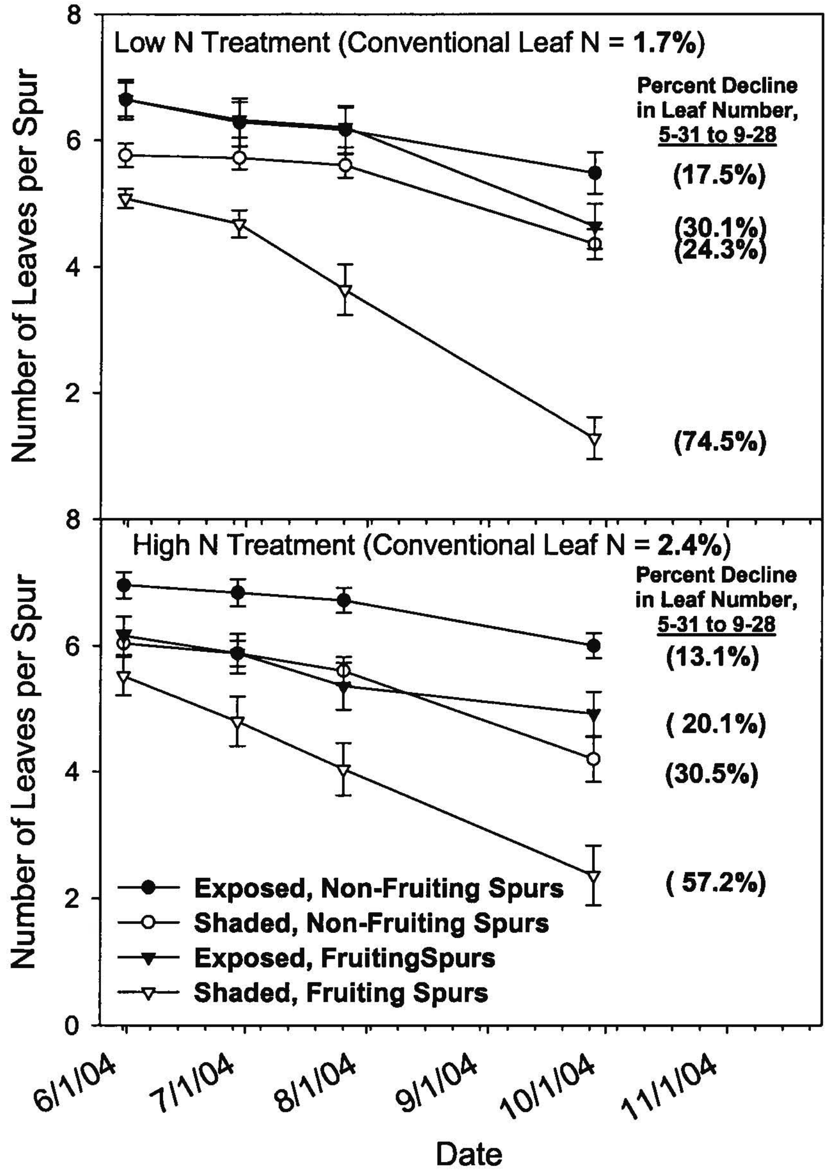

Figure 1. The pattern of leaf drop, May 31 to September 28, 2004, for spur sub-populations differing in fruiting status and light exposure. Data are means ± standard error.

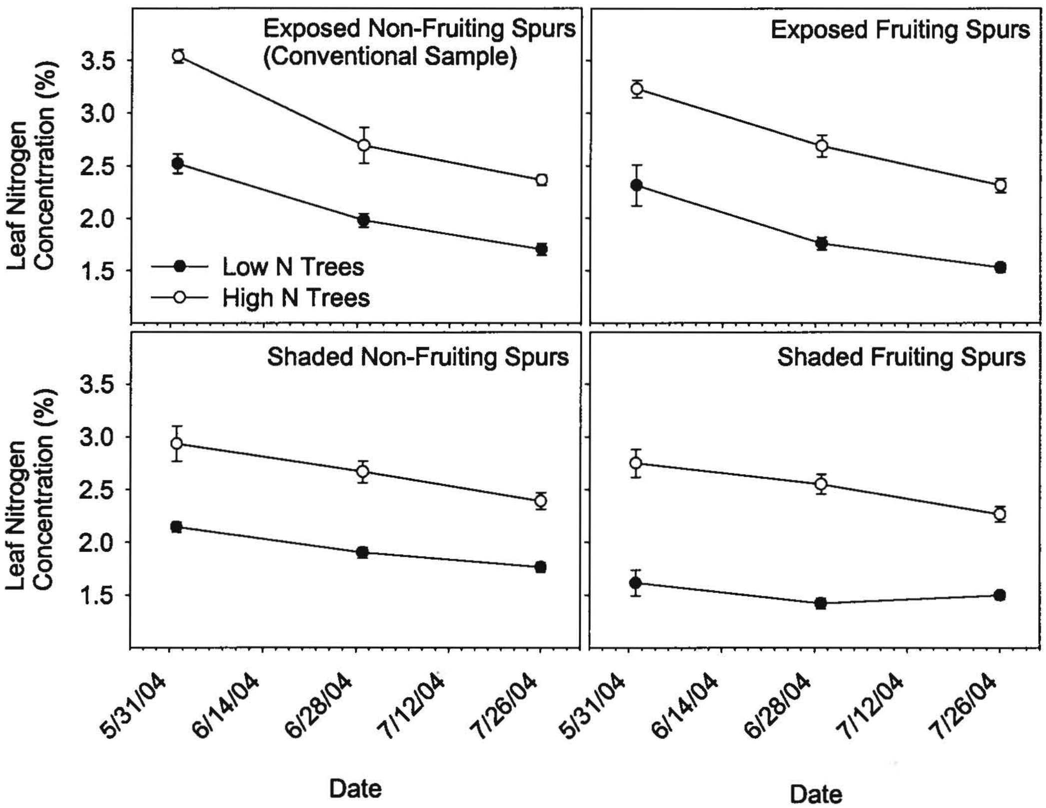

Figure 2. The pattern of change in nitrogen (N) concentration of leaves sampled from four almond spur sub-populations during the period of embryo development (June 1 to July 26, 2004). Data are means ± standard error.