The effect of plant nutrition on pollen production and composition

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Problem and its significance

The success of our agricultural economy depends on abundant crop production with a minimum investment of labor and materials. In many cases, efficient and thorough pollination is a key element affecting both crop yield and quality. Most commercial almond cultivars require cross-pollination when flowering occurs in early spring. At no small expense, colonies of the honey bee, *Apis mellifera,* are often brought into the orchard to facilitate cross-pollination between individual cultivar types. However, the period of time when flowers are receptive to pollination is short (Griggs 1969), and often the weather conditions during the spring are unfavorable for pollinator flight (Dag et al. 2000). It is important, then, to maximize the efficiency with which honey bees work an orchard. Understanding the factors affecting bloom and pollen attractiveness to honey bees can translate to better pollination and improved product yield and quality.

Essential for the viability of pollen is the secondary metabolite sulfur, though it is often overlooked in nutritional planning. Recent discoveries in plant science have identified a number of small sulfur-containing compounds with biological significance in plants. The role these compounds may play in the plant include: counteracting oxidative stress and ozone exposure (glutathione), mediating fungal attack and bacterial disease (thionins and definsins), and detoxifying environmental pollutants (phytochelatins.) Of particular interest are defensins, which are small proteins found throughout nature that are active components of the host defense

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system. First isolated in plants in 1995 (Terras et al. 1995), they are often found associated with seeds, fruit, flowers, and stamen of a wide variety of plants (Meyer et al.1996).

How nutritional programs influence the production of sulfur rich compounds such as defensin is not clear, but recent research[3] suggests that sulfur nutrition is not always optimal and plants may have to deal with temporary or prolonged periods of sulfur stress.
Nor has the relationship of sulfur nutrition to pollen production and composition been described in the literature. However, experiments done by us indicate that sulfur nutrition has a measurable and significant effect on pollen production in certain flowers with as much as a doubling of flower head weight but with minimal changes in green weight values. The strength of these initial results, coupled with the new research on sulfur deficiency and sulfur induced resistance, and the potential impact changes in pollen composition may have on plant health, pollination success, and crop yield begs the question whether simple and rational changes in plant sulfur nutrition can help guide better pollen production and improve crop yield and quality.

Objectives

The primary goal of this research was to evaluate the effect of sulfur fertilization on pollen production and characterize any changes in pollen composition. If differences were detected, then a second goal of this research was to evaluate whether bees would show a preference for pollen produced by trees grown under high versus low sulfur fertilization.

Methods

Field Conditions We had the fortunate opportunity to come in at the end of a 20 acre field trial underwritten by Best Sulfur Products that was designed to evaluate the performance of the sulfur-containing fertilizer calcium thiosulfate (Thiocal™) on almond cultivars Butte and Padre. The trial was conducted in Modesto, CA, through Ripon Farm Supply under the direction of Frances Gruen. The treatment was applied in 2003 at a per acre rate of 10 gal at bud

[3]See, for example, the Cost Action 829: Fundamental, Agronomical and Environmental Aspects of Sulfur Nutrition and Assimilation in Plants http://cost829.dhs.org/general_information

break, 10 gal at petal fall, 10 gal mid season and 10 gal just before nut hardening. We collected pollen during the bloom in 2004.

Pollen collection Pollen was collected from both treated and untreated Padre and Butte cultivars. Samples were collected on February 27, 2004, just as the trees began to blossom. Small branches containing an average 5-8 flowers that were nearly open were cut front the trees and placed in plastic bags. Samples were collected in a single afternoon by two researchers. The bags were stored on ice and shipped overnight express mail from Modesto to the Tucson laboratory. Upon receipt, the bags were weighed, and the flowers removed and placed on white paper where the blooms continued to open. After about an hour most of the flowers had opened and the anthers had dehisced (Figure 1). Debris and unopened flowers were removed and the remaining anthers were gently rubbed and tapped to drop the pollen onto the paper. Each bag of approximately 400 grams of flowers and stems yielded a total of 0.5 gms pollen +/- 10%.

Fig 1. Flowers with petals removed prior to pollen collection.

Amino Acid Analysis The distribution of amino acids was performed by an outside laboratory[4] specializing in amino acid analysis and equipped with Beckman Instruments Models 6300 and 7300 amino acid analyzers. Pollen samples were prepared for analysis by treatment with hydrochloric acid to free constituent amino acids from total contained protein. The acid hydrolysis preparation method is not reliable for tryptophan, nor for the sulfur amino acids cysteine and methionine. Cysteine and methionine were prepared separately by performic acid digestion. Tryptophan was obtained by caustic hydrolysis. Absorbance was measured at 440 and 570 nm following post-column color development by Ninhydrin reagent at 131 degrees C.

Volatile Analysis The volatile floral profiles of the pollen were collected using Solid Phase Microextraction (SPME) techniques and analyzed by GC/MS. Samples of pollen were collected by placing 250 mg pollen into a 2 ml vial. A SPME fiber (65µm polydimethylsiloxane/divinylbenzene; Supelco 57326U) was inserted in the vial for 10 minutes,

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being careful not to touch the pollen itself. The collected volatiles were then analyzed by GC/MS (Varian CP-3800, Saturn 2200 Ion Trap MS). The fiber was desorbed for 3 minutes and compounds separated on a Varian VF-5MS 30m x 0.25 ID column with the following programmed parameters: injector temperature 240°C; column temperature 40°C for 3 min, ramp to 250°C at 20 degrees per minute; flow rate 1 ml/min. The MS was operated in EI mode at 150 eV.

Results and Discussion

Tissue samples and yield data from the field trial indicated that there were no gross differences between treated and untreated almond cultivars that were statistically significant (Faith Potter, personal communication). It was suggested that results of fertilizer treatment might not show up on some permanent crops until the second or third year. It is also possible that sulfur stores were already optimal for the plant. Since sulfur plays such an important role in plant defense mechanisms, the advantages of supplementation, or the losses due to deficiencies, sometimes do not show up until the crop is stressed by disease or water shortage (Tom Fairweather, agronomist, personal communication). The grower did report that treatment improved water penetration. This effect was significant enough to warrant commitment to another series of applications for the 2005 crop. We hope to sample pollen again next year to see if the difference we expected are more dramatic.

The amino acid analyses are shown in Table 1 and are reported as mg of each amino acid per gram of pollen. Two differences may be noted. First, the effect of treatment on the Butte cultivar is different than the Padre cultivar. On average, the treated Butte pollen showed a consistent reduction in pollen quantity for all amino acids (-22.5 +/- 0.82%). The Padre cultivar showed no such consistency in reduction but rather showed a wide range of changes, from –3.7% reduction (methionine) to a 56.9% increase (tyrosine), and an overall increase in amino acids by around 5.6%.

Second, both cultivars show a mild reduction in nearly all sulfur amino acids *with* sulfur fertilization. This suggests that feedback mechanisms may have been activated, shifting the distribution of sulfur compounds as a result of increased sulfur stores. Sulfur metabolism in

plants is fairly complex, but in general it is either assimilated through a series of reductions ultimately resulting in the synthesis of the amino acid cysteine, or it may be directly incorporated into organic molecules though the intermediate PAPS (3'-phosphoadenosine 5'-phosphosulfate) forming sulfhydral groups and sulfated compounds. The amino acids cysteine and methionine may also be incorporated into the production of a variety of other compounds. The possibility of the activation of feedback mechanisms by the addition of sulfur fertilization cannot be ruled out, but pursuing that possibility was beyond the scope of this study.

In the absence of treatment-induced changes to the growth habit of the two cultivars, it is difficult to draw conclusions about the effect of fertilization. But the real test – the production of almonds – has yet to be realized. It will be interesting to see if yield data from the upcoming Fall 2004 harvest reflects any observations we've made regarding the amino acid analysis. Long term storage and resistance to disease will also be of interest since sulfur is known to affect both these properties.

Table 1. Amino Acid analysis of pollen from Butte and Padre Cultivars, in mg per gm pollen sample.

amino acid	Butte U	Butte T	% change	Padre U	Padre T	% change
L-Aspartic acid	49.67	40.32	-18.8	42.87	44.89	4.7
L-Threonine	17.78	12.79	-28.0	14.34	15.88	10.7
L-Serine	15.79	11.82	-25.2	13.17	13.77	4.5
L-Glutamic acid	34.26	26.46	-22.8	31.30	32.64	4.3
L-Proline	41.26	32.16	-22.0	42.05	43.78	4.1
L-Cysteine *	6.43	5.54	-13.7	6.43	6.76	5.1
L-Glycine	19.99	15.38	-23.1	18.26	18.91	3.6
L-Alanine	23.71	18.39	-22.4	21.86	22.50	3.0
L-Valine	26.01	20.75	-20.2	23.58	24.54	4.1
L-Methionine *	10.34	8.46	-18.2	11.21	10.79	-3.7
L-Isoleucine	19.97	15.30	-23.4	18.81	19.35	2.8
L-Leucine	31.30	24.11	-23.0	28.41	29.53	3.9
L-Tyrosine	11.74	8.36	-28.8	6.80	10.66	56.9
L-Phenylalanine	21.25	17.05	-19.8	16.76	18.27	9.0
L-Histidine	10.52	8.07	-23.2	9.04	9.98	10.4
L-Lysine	33.52	26.30	-21.5	30.80	32.37	5.1
Ammonia	3.76	2.74	-27.1	2.95	3.17	7.7
L-Arginine	23.61	17.69	-25.1	18.37	19.54	6.4
L-Tryptophan**	4.11	3.22	-21.8	3.50	3.48	-0.5
Total amino acids	405.00	314.92	-22.2	360.49	380.81	5.6

* Denotes sulfur-containing amino acid

Using SPME techniques, the volatile profile of the treated and untreated pollen samples were collected and compared for any evidence that treatment had an effect on the volatile constituents of the pollen. Figure 2 shows that all four samples – Butte and Padre treated and untreated – had very similar volatile profiles or "fingerprints". Both the number and location of peaks, and their relative proportion to each other, appears the same between the four samples. Tentative identifications along with % closeness of fit to library standards are provided in the caption. Intriguingly, while the fingerprints were similar, the intensity level was higher in both of the Thiocal treated cultivars compared to the untreated samples. These differences are more clearly evident in the comparison of the area of peaks 1-7 given in Table 2, which shows an increase in all compounds as a result of treatment, with some very dramatic increases in the Padre cultivar for peaks 1, 5, 6 and 7.

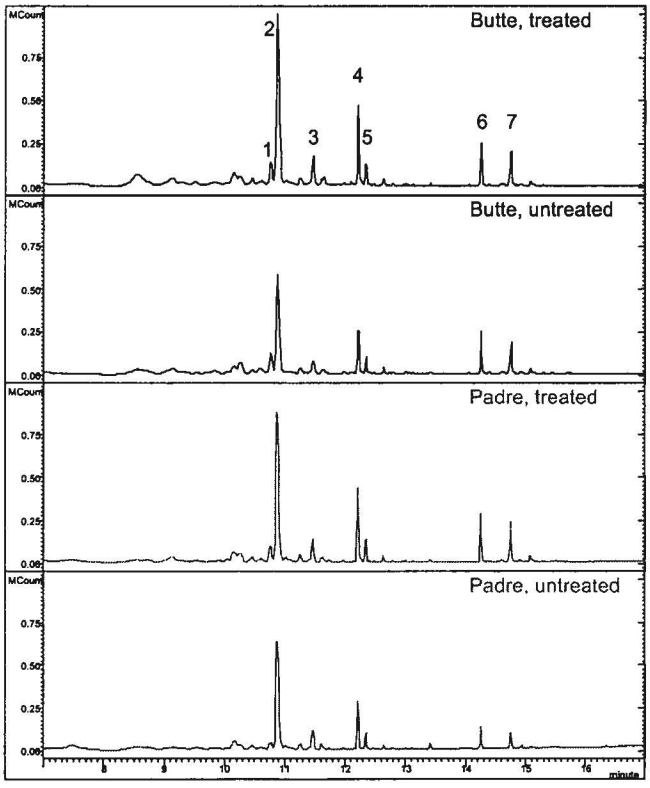

Fig. 2 Chromatogram of the volatiles from Thiocal treated and untreated Butte and Padre cultivars.

Tentative identification of the seven major peaks is given below, with percent "fit", or match, to Wiley library standards:

1 β-citronellol, 90%

2 3,5-dimethyl-cyclohexanol, 73%

3 Z-9-octadecenal, 83%

4 β-cyclocitral, 92%

5 hydroxycitronellal, 73%

6 α-ionone, 92%

7 β-ionone, 92%

Table 2 The change in area under the peak for peaks 1-7 as a function of Thiocal treatment

Peak	Butte T	Butte U	% Change	Padre T	Padre U	% Change
1	432.96	400.55	8.1%	297.95	129.31	130.4%
2	3864.83	2238.40	72.7%	3275.16	2433.72	34.6%
3	518.13	223.69	131.6%	392.83	309.63	26.9%
4	899.38	541.65	66.0%	751.22	517.08	45.3%
5	311.57	196.49	58.6%	292.26	92.66	215.4%
6	400.19	338.44	18.2%	389.62	176.60	120.6%
7	446.96	371.67	20.3%	463.64	184.26	151.6%
Total	6874.02	4310.89	59.5%	5862.68	3843.27	52.5%

Though not originally in the scope of this project, we also sampled the volatiles emitted from the almond flowers. Here we were surprised to discover that not only were the compounds represented in higher amounts in the treatment sample, but also an entirely new peak was present. Figure 3a shows the profile of Padre treated and untreated flowers. Using the fragment pattern of benzaldehyde – the compound that gives the characteristic odor to almonds – as a selection tool, the difference between the two treatments is evident (Figure 3b).

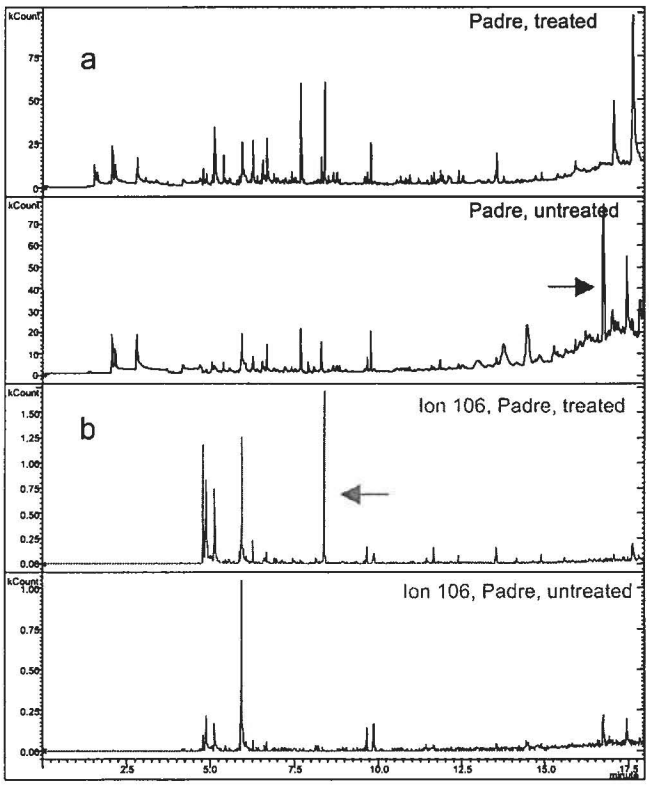

Figure 3. Chromatogram of Padre treated and untreated flowers.

Section a shows the raw chromatogram while section b shows the pattern when fragments specific to benzaldehyde-type compounds are selected.

Note the difference in high molecular weight compound in section a (arrow). These are mostly saturated alkanes but also include 1,2 benzene dicarboxylic acid butyl phenyl methyl ester (tentative ID) in the untreated Padre sample.

The arrow in section b, Padre treated sample, points to an unknown benzaldehyde-type substance that is not present in the untreated sample.

Given the parameters of the field trial, it is not possible to differentiate whether the difference in volatile profile between treated and untreated samples is due to the calcium or the sulfur that is provided by Thiocal fertilization, or is simply due to the increased water penetration provided by cation exchange with calcium in the soil. Future trials might include comparison with gypsum, calcium chloride, and/or soil sulfur to tease apart the effects of the calcium cation from the thiosulfate anion. It will be very interesting to note whether or not the change in volatile profile evidenced here can be correlated with changes in yield or other properties that would indicate better pollination. It would not be unreasonable to expect that a larger volatile signal might attract more bees to the source. It is also possible that the improved water penetration produced by Thiocal treatment might increase nectar production. This, too, would increase the attractiveness of the bloom to the bees.

The results of this study are intriguing, and the effect of odor is surprising. We suggest that this research be continued in the 2005 bloom season with measurements for nectar production and bee visitation to establish whether treatment improves overall attractiveness. We also suggest monitoring for changes in disease resistance, yield and storage properties of the 2004 harvest.

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