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Efficacy of foliar-applied cytokinins and nitrogen to increase floral bud retention to reduce alternate bearing of pistachio

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NB. This research paper was published in 2005. In the reprint below three sections have been omitted - Abstract, Materials and Methods, and Results including supporting data. However the full version of this research paper can be viewed by going to the website: www.actahort.org/books/727/727_42.htm Australian readers must be mindful that the months quoted apply to northern hemisphere seasons. It must also be noted that the research was undertaken on the pistachio cultivar 'Kerman'. Ed.

Introduction

Alternate bearing, the production of a heavy on crop followed by a light off crop, is a problem of increasing economic significance to the California pistachio industry. Not only does it result in price instability and uneven annual returns to growers, it can result in a loss of market share in the off-crop year that is not always regained the following year, and it also prevents the development of new value-added products.

Alternate bearing can be initiated by climatic conditions that result in a low-yielding off-crop year, such as insufficient chilling that results in a low number of flowers at bloom, or temperature extremes during flowering, fruit set or June drop that result in excessive flower or fruit abscission. Conversely, when these factors are optimal and little flower or fruit abscission occurs, a heavy on-crop results. For pistachio (*Pistacia vera* cv. Kerman), the mechanism by which fruit number one year influences the return bloom and yield the next year was identified more than 30 years ago (Crane and Nelson, 1971). During the on-crop year, excessive abscission of floral buds beginning with the initiation of embryo growth in June and intensifying during the period of rapid embryo growth (nut fill) in July, results in the next year's off crop. Whereas the mechanism perpetuating alternate bearing in pistachio is known, the physiological basis underlying the mechanism remains unresolved. There is convincing evidence that the floral buds fail to compete successfully against the developing fruit for available carbohydrates, and thus abscise (Crane and Nelson, 1971, 1972; Crane et al., 1973; Crane and Iwakiri, 1987). However, Crane et al. (1976) provided results that were inconsistent with the tenet that carbohydrate is limiting during an on-crop year. Incrementally increasing the number of fruit removed from shoots carrying an on crop failed to increase the concentrations of carbohydrate available in the floral buds or the subtending shoots during the abscission period but increased floral bud retention proportionally to the number of fruit removed. This suggested the possibility of a hormonal basis for the perpetuation of alternate bearing in pistachio. Crane et al. (1973) proposed that the on crop resulted in the loss of a root or leaf synthesized "anti-abscission" hormone or in the accumulation in the floral buds of a fruit-produced abscission-promoting hormone. Takeda and Crane (1980) provided evidence that cis-trans-abscisic acid (ABA) accumulated in floral buds during the period of rapid increase in seed growth during June through July. However, the authors concluded that ABA was not the causal factor since floral buds also abscised prior to this period and no ABA accumulation in the buds had occurred. In addition to a strong demand for carbohydrate during the on-crop year, Weinbaum et al. (1994a, b) provided evidence that in an on-crop year there is a strong reproductive demand for nitrogen, significant removal of nitrogen in the fruit at harvest, reduced storage of nitrogen, reduced recovery of January-applied ^{15}N fertilizer, and greater root nitrate concentrations (the latter possibly due to greater uptake or reduced assimilation and transport to other parts of the tree). It is of interest that these authors observed the greatest decrease in leaflet nitrogen concentration and total leaflet nitrogen content per tree during the period from rapid embryo development (nut fill) (early July) through fruit maturation (early September) and that nitrogen removed by the harvest of mature fruit plus the loss of senescent leaflets was 1.0 kg N per tree during an on-crop year versus only 0.2 kg N per tree in an off-crop year. Frequently, leaflets at the base of fruit clusters show early senescence in on-crop years (L. Ferguson, personal communication). Premature senescence, which can be due to nitrogen deficiency, would cause a further loss in photosynthesis, carbohydrate availability, and leaf-produced hormones, as well as essential soluble nitrogen compounds.

In the research presented here, we focused on the role of cytokinins and nitrogen in alternate bearing of pistachio. We quantified the endogenous concentrations of zeatin-riboside (ZR) and isopentyladenosine (IPA) in floral buds collected from on-crop trees (> 70 fruit per cluster at the base of the shoot bearing the buds for next year's crop). Based on the results of this analysis, which revealed that the cytokinin concentrations of buds decreased as embryo growth progressed, we tested the efficacy of the cytokinin 6-benzyladenine (BA) (MaxCel®, Valent BioSciences Corp.) and an algal extract from *Ascophyllum nodosum* (Binary CQ®, Helena Chemical Co.) combined with low-biuret urea or N-P-K to reduce floral bud abscission during the on-crop year and, thus increase yield the following, putative off-crop year. A preliminary 2-year study compared the efficacy of the two cytokinins in the same orchard. A second field experiment of 5 years duration was conducted to further test the efficacy of the algal extract in combination with a higher rate of low-biuret urea to reduce alternate bearing and increase cumulative yield. The higher rate of low-biuret urea

was tested with the additional goal of using foliar-applied N to replace soil-applied N to reduce the potential for pollution of groundwater with nitrate. A successful strategy using an algal extract cytokinin source to increase yield would prove of value as an alternative to 6-benzyladenine, especially for the increasing number of organic pistachio growers of California.

Discussion

The results of this research provide evidence that floral bud abscission in 'Kerman' pistachio is related to reduced cytokinin concentrations in the floral buds. Concentrations of both ZR and IPA decreased 40% from the initiation of embryo growth in June to early in the period of rapid embryo growth in July. Cytokinins are well known for their role in maintaining sink strength and in preventing senescence. Reduced cytokinin concentrations would likely contribute to "weakening" the sink strength of floral buds making them less able to compete for resources against the developing fruit. In addition, low cytokinin concentrations in the floral buds might contribute to their senescence and, hence, abscission. It was originally presumed that the heavy on-crop reduced the amount of nitrogen available to leaves causing early leaflet senescence and a loss of cytokinins or similarly that reduced nitrogen availability to the roots might have compromised their ability to provide sufficient amounts of cytokinins to meet the needs of both the fruit and floral buds. However, more recent results (Verryenne, 2005) suggest that the fruit might simply be a stronger sink than buds for cytokinins moving in the xylem. For the 'Pixie' mandarin (*Citrus reticulata* Blanco), apical buds on fruit-bearing shoots of on-crop trees had low IPA concentrations. Removal of the fruit increased the IPA concentration of the apical buds.

Our results showing a loss of cytokinins in floral buds during the period of floral bud abscission taken together with those of Weinbaum et al. (1994a, b) demonstrating the significantly greater amount of N removed by on-crop trees provided the basis for foliar strategies combining cytokinins and N to increase floral bud retention during the on-crop year to increase yield the following year (potential off-crop year). In addition, it was presumed that this strategy would reduce the early leaflet senescence observed in on-crop years and, in turn, would increase the export of metabolites, including carbohydrates, nitrogen compounds and hormones, to the developing fruit and floral buds. Consistent with our intent, floral bud abscission of 'Pontikis' pistachio was recently shown to be negatively correlated with floral bud concentrations of polyamines (Roussos et al., 2004). Our approach gained support by the fact that all treatments using cytokinin as BA or algal extract combined with N as low-biuret urea or N-P-K fertilizer tested in the present study increased bud retention 1.6- to 2.0-fold on shoots bearing greater than 70 fruit per cluster on on-crop trees compared to untreated control shoots. When these treatments were adjusted and applied to whole trees, as anticipated, the treatments had no effect on yield in the on-crop year (first year applied) but increased yield the following year (putative off-crop year) such that all treatments significantly increased 2-year cumulative yield as kg fruit (fresh wt) per tree and kg split nuts (dry wt) per tree compared to the untreated control trees. However, despite the significant positive effect of the treatments, off-crop yields as kg fruit (fresh wt) per tree and kg split nuts (dry wt) per tree remained 70% and 80% lower, respectively, than the on-crop yields attained in year 1 in this orchard. The ABI (based on kg fruit [fresh wt] per tree) for the 2 years of the study improved from 0.78 for the untreated control trees to 0.58 for trees treated with BA (69 g) plus 6.9 kg N as low-biuret per ha per application at the initiation of embryo growth (June) and early in the period of rapid embryo growth (July). This treatment, although the best had a less positive effect on ABI based on kg split nuts (dry wt) per tree, the commercially important parameter, reducing ABI to 0.72 compared to 0.84 for the control trees.

The algal extract and N treatment used in the 5-year yield

experiment did not prove as efficacious as the applications of algal extract and N used in the 2-year yield experiment. Foliar application of 4.7 L algal extract combined with 28 kg N as low-biuret urea per ha in June and again in July failed to significantly increase the 5-year cumulative yield of split nuts (dry wt as kg/tree). Whereas the treatment significantly reduced ABI in 2 of 5 years compared to the untreated control, it did not significantly reduce the 5-year average ABI. However, the treatment significantly increased the cumulative yield for the three off-crop years as both kg fruit (fresh wt) per tree ($P = 0.004$), but not the cumulative yield of the on-crop years. This result suggests that the treatment was increasing floral bud retention in the on-crop year. Because the average 12.2 kg net increase in fruit (fresh wt) per tree for the three off-crop years compared to the control resulted in an average 10.3 kg net increase in split nuts (dry weight) per tree for the three off-crop years, it is unlikely that the algal extract plus N treatment simply increased hull (exocarp and mesocarp) weight of the fruit in the off-crop years.

The exact cause of the reduced efficacy of the algal extract plus N treatment in the 5-year experiment is unknown. Increasing the algal extract from 2.4 to 4.7 L ha-1 and N from 6.9 kg to 28 kg ha–1 as low-biuret urea and reducing the number of applications from May, June and July to just June and July, likely contributed to the compromised efficacy of the algal plus urea strategy used in the 5-year study compared to the 2-year study. Phytoxicity was evident as tip burn of the leaflets on some trees following treatment application in some years. Whether this was due to ammonia toxicity resulting from the high rate of low-biuret urea applied or due to the increased amount of biuret the trees received from the high application rate and the change from Unocal PLUS liquid urea with < 0.1% biuret to granular urea with 0.25% or both is unknown. The possibility that the grower applied N to the soil near the time of the first treatment application in some years cannot be ruled out. In addition, in some years there would have been less than 1 month between the two applications due to a faster rate of embryo development in warmer years. In year 1 of the experiment, the algal extract plus low-biuret urea treatment caused the greatest amount of leaflet tip burn and the yields of fruit (fresh wt) and split nuts (dry wt) as kg per tree were both numerically reduced compared to the untreated control. In the four subsequent years, the treatment had a consistent positive effect on yield resulting in a significant 4-year cumulative net increase in kg fruit (fresh wt) and kg split nuts (dry wt) per tree ($P = 0.01$ and $P = 0.08$, respectively). In subsequent research, foliar application of 14 kg N as low-biuret urea (0.25% biuret) in 934 L ha-1 water or 26.2 kg N in 1869 L ha-1 water, caused significant leaflet burn of 'Kerman' pistachio; some leaflet burn was obtained with 26.2 kg N as low-biuret urea in 2804 L ha-1 water (Lovatt, unpublished).

A question that arose during this research was whether treatments should be applied only during the on-crop year or annually. The results of the research presented herein argue for annual application to compensate for unanticipated climatic events that reset the alternate bearing cycles, in this case lack of chilling during the winter that resulted in a weak bloom and two sequential off-crop years. Moreover, the third year of the study, though an off-crop year by industry standards, was not as low a yield as the other two off-crop years.

The results of the bud retention and two yield experiments, although preliminary in nature, suggest that in an alternate bearing pistachio orchard an algal extract cytokinin source combined with low-biuret urea can be used as foliar treatment to increase floral bud retention in an on-crop year to improve the yield of split nuts (kg dry wt). It is clear that the algal extract (and BA) and N strategies tested in the 2-year yield experiment should be investigated further and that the rate of low-biuret urea used in the 5-year yield study needs to be reduced. Given that pistachio growers in California prefer to apply foliar treatments in only 934 L ha-1 water, low-biuret urea would need to be reduced to less than 14 kg N ha-1. Development of a successful strategy to increase floral bud retention, reduce alternate bearing and increase cumulative yield using the algal extract with an appropriate source of nitrogen is of great interest to the organic pistachio growers of California.

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