Efficacy of Foliar-applied Cytokinins and Nitrogen to Increase Floral Bud Retention and to Reduce Alternate Bearing of Pistachio

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Abstract

Alternate bearing in pistachio (*Pistacia vera* L., cv. Kerman) is due to excessive abscission of floral buds for next year's crop during the heavy on-crop year. Floral bud abscission begins with the initiation of embryo development (June) and proceeds through the period of rapid embryo growth (July). During this period, zeatinriboside and isopentyladenosine each decreased 40% in floral buds on shoots of on-crop trees (more than 70 fruit per shoot). Foliar-applied 6-benzyladenine (BA) (25 or 50 mg L^{-1}) with low-biuret urea (0.25% N) per application in June and July or May (early fruit set), June and July increased bud retention on shoots of on-crop trees ≥ 1.6-fold compared to bud retention on untreated shoots (*P* ≤ 0.05). Three applications of an algal extract from *Ascophyllum nodosum* (0.08%) with low-biuret urea (0.25% N) or N-P-K (11-8-5) liquid fertilizer (0.17%) in May, June and July were equally effective, increasing bud retention 1.9- and 2.0-fold, respectively. In commercial orchards, the efficacy of 69 g BA plus 6.9 kg N as low-biuret urea per ha per application and 2.4 L algal extract plus 6.9 kg N as low-biuret urea or N-P-K fertilizer at 4.7 L per ha per application to increase bud retention during the on-crop year and increase yield the following (off-crop) year was determined. Treatments were initiated during the on-crop year and applied annually in June and July or in May, June and July. All treatments increased 2-year cumulative yield (kg split nuts [dry wt] per tree) (*P* = 0.0018). In a subsequent 5-year experiment, the algal extract at 4.7 L plus 28 kg N as low-biuret urea per ha per application in June and July increased the cumulative yield of kg split nuts (dry wt) for the three off-crop years (*P* = 0.10), but not the 5-year cumulative yield (*P* = 0.12). The algal extract combined with low-biuret urea had a significant but variable effect on alternate bearing index. No treatment affected nut quality. There is great interest in improving the efficacy of the algal extract paired with an appropriate nitrogen source for use in organic pistachio production.

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 a value-added products industry.

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 and isopentyladenosine 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.

MATERIALS AND METHODS

The initial research was conducted in a commercial orchard of 17-year-old 'Kerman' pistachio on Pioneer Gold I (*Pistacia integerrima*) rootstock (292 trees/ha) in

Madera, Calif. (36.57° N, 120.03° W). This included quantification of the cytokinin content of floral buds from on-crop trees, a branch study the following year testing the efficacy of cytokinin and nitrogen treatments to increase floral bud retention during the on-crop year, and a 2-year field experiment testing the efficacy of the same treatments used in the bud retention study to increase yield in the off-crop year and, thereby, increase cumulative yield.

A 5-year field experiment was conducted to test the efficacy of the algal extract cytokinin combined with low-biuret urea to increase yield in the off-crop year in a commercial orchard of 32-year-old 'Kerman' pistachio on Atlantica (*Pistacia atlantica*) rootstock near Kettleman City, Calif. (36.01° N, 119.96° W). The orchard was planted at 262 trees per ha. The trees were pruned as needed every other year.

In both orchards, data trees were selected for uniform health, size, vigor and crop load. Treatments were an addition to each grower's standard production practices. Both orchards were monitored over time to keep track of the occurrence of spring bud break, full bloom and embryo development for the purpose of timing research activities and treatment applications.

Floral Bud Cytokinin Concentration

Floral buds (50) for next year's crop were collected from shoots with more than 70 fruit per cluster from five replicate on-crop trees at the initiation of embryo development (14 June) and during the period of rapid embryo growth (nut fill) (6 July). Endogenous concentrations of zeatinriboside (ZR) and isopentyladenosine (IPA) were quantified by radioimmunoassay (RIA).

Three replicate samples (500 mg dry weight) from each freeze-dried and ground bud sample were extracted in 40 mL of cold 80% methanol in a 50-mL Erlenmeyer flask overnight at 4 °C. Each extract was filtered through Whatman #1 filter paper, the filtrate was transferred to a rotary evaporation flask, and ^{14}C-IAA (~ 4000 dpm; specific activity of 57 mCi/mmole, Sigma) was added to each sample as an internal standard to quantify losses during purification. Purification and analysis of the samples were performed as described previously (Bohner and Bangerth, 1988a, 1988b) with modifications (Bertling and Bangerth, 1995). Following the removal of methanol using a rotary evaporator, the extracted samples (adjusted to pH > 8.5 with 0.1 M ammonium acetate) were applied to a 5.5-mL polyvinylpolypyrrolidone (PVPP) column, below which was attached a 5-mL [2-(diethylamino) ethyl-Sephadex®] (DEAE) anion exchange column. The tandem columns were eluted with 30 mL of 0.01 M ammonium acetate (pH 8.0). Cytokinins were collected in a Sep-Pak C18® cartridge (Waters Corp., Milford, Mass.) attached to the end of the DEAE column and eluted with methanol.

Polyclonal antibody production, for use in RIA, included the coupling of each hormone to bovine serum albumin and the subsequent inoculation of rabbits with the coupled conjugate to produce antisera (Cutting et al., 1983). Tracer compounds consisted of ^{3}H-labeled ZR and IPA and were obtained from Amersham Life Science, Inc., Arlington Heights, Ill. and Sigma, St. Louis, Mo., respectively. All immunoassays were performed in triplicate using the method described by Cutting et al. (1983). Hormone concentration was calculated from the measured radioactivity of the bound phase using the spline approximation method and was reliable from 0.01 ng 100 μL^{-1} to 50 ng 100 μL^{-1} for each of the hormones analyzed. Samples were diluted to obtain concentrations in this range. Recovery of the ^{14}C-IAA internal standard was used to correct for loss.

Floral Bud Retention

The following treatments were tested for their ability to increase floral bud retention: (*i*) untreated control; (*ii*) 6-benzyladenine (BA) (MaxCel®, Valent BioSciences Corp.) (25 mg L^{-1}) with low-biuret urea (Unocal PLUS®, Unocal Corp., 20% N, < 0.1% biuret) (0.25% N) applied 12 June and 8 July; (*iii*) BA (25 mg L^{-1}) with low-biuret urea (0.25% N) applied 12 May, 12 June and 8 July; (*iv*) BA (50 mg L^{-1}) with low-biuret urea (0.25% N) applied 12 May, 12 June and 8 July; (*v*) algal extract (Binary CQ®, Helena

Chemical Co.) (0.08%) with low-biuret urea (0.25% N, < 0.1% biuret) per application on 12 May, 12 June and 8 July; and (*vi)* algal extract (0.08%) with N-P-K liquid fertilizer (11-8-5, Bayfolan®, Helena Chemical Co.) (0.17%) applied 12 May, 12 June and 8 July. Each treatment (adjusted pH 5.5) was applied to the foliage of a single shoot bearing more than 70 fruit in a randomized complete block design on each of 16 on-crop trees with a backpack sprayer to the point of run-off. Application times corresponded to fruit set (12 May), initiation of embryo growth (12 June) and start of rapid embryo growth (8 July). The May application was included to preload the buds with cytokinin and N prior floral bud abscission. Initial number of fruit and initial number of floral buds for next year's crop per shoot were counted 1 day prior to treatment in May and again in June. Final fruit and floral bud number per shoot were counted just before harvest (4 Sept.).

Yield Experiments

The treatments above were adjusted for application to whole trees and tested on 16 individual tree replications in a randomized complete block design for 2 years. Treatments per ha were: (*i*) untreated control; (*ii*) BA (69 g) with 6.9 kg N as low-biuret urea applied in June and July; (*iii*) BA (138 g) with 6.9 kg N as low-biuret urea applied in May, June and July; (*iv*) algal extract (2.4 L) with 6.9 kg N as low-biuret urea applied May, June and July; and (*v*) algal extract (2.4 L) with 4.7 L N-P-K liquid fertilizer applied in May, June and July. Treatments were applied in 2804 L ha^{-1} water (adjusted to pH 5.5) with a 2.76 MPa handgun sprayer to the point of run-off on dates corresponding each year to fruit set (early to mid-May), initiation of embryo development (early to mid-June) and at the beginning of the period of rapid embryo growth (early to mid-July). Treatments were initiated in the on-crop year and applied annually.

In the 5-year experiment, an untreated control was compared to the algal extract at 4.7 L combined with 28 kg N as low-biuret urea (granular 46% N, 0.25% biuret) per ha applied to the foliage annually at the initiation of embryo development (early to mid-June) and again at the beginning of the period of rapid embryo growth (early to mid-July) beginning in the on-crop year. There were 10 trees per treatment with fourteen replications in a randomized complete block design. The treatment was applied in 2506 L of water (pH 5.5) per ha, which was sufficient to provide full canopy coverage to the point of run-off, using a handgun sprayer at 2.76 MPa.

For both experiments, commercial shaking and catching equipment was used to harvest the trees. Yield (kg fruit/tree) was determined in the field using portable scales. Subsamples (100 fruit/tree) were collected, and the seed (nut) was removed from each fruit. The following nut quality parameters were determined: nut fresh weight, dry weight (5% moisture content), percent split versus unsplit nuts, nuts with aborted embryos and blank nuts (no evidence of embryo growth).

The severity of alternate bearing is expressed as the alternate bearing index (ABI). ABI was calculated for each sequential 2-year period using the equation: ABI = (year 1 yield minus year 2 yield) / (sum of year 1 yield plus year 2 yield), where yield is in kilograms fruit per tree. When ABI = 1.0, there is complete alternate bearing, i.e., crop one year with no crop the second year. An ABI of zero means alternate bearing is absent.

Statistical Analyses

Data for both the branch and yield experiments were analyzed using the General Linear Model procedure of the SAS statistical program (SAS Inst., Inc., Cary, N.C.). Analysis of variance was used to test treatment effects on annual yield, 5-year cumulative yield, cumulative on-crop and off-crop yields, alternate bearing index, average nut dry weight, percent splits and percent blanks. Means were separated using Duncan's multiple range test at $P = 0.05$ and $P = 0.10$. Note that for cumulative yields, a missing datum point for a tree in any year excluded all the data for that tree from the statistical analysis.

RESULTS

Floral Bud Cytokinin Concentration

The cytokinin concentrations of floral buds collected from on-crop trees decreased during the early period of floral bud abscission (Table 1). Concentrations of ZR and IPA in floral buds collected at the initiation of embryo development (14 June) were both 40% lower 3 weeks later at the beginning of exponential embryo growth (6 July) ($P = 0.07$ and $P = 0.06$, respectively).

Floral Bud Retention

More than 70% of the floral buds on untreated shoots (control) of on-crop trees abscised by harvest (4 Sept.) (Table 2). All treatments significantly increased bud retention on shoots of on-crop trees compared to control shoots, which retained only 2.5 buds per shoot ($P = 0.05$) The algal extract plus low-biuret urea or N-P-K applied in May, June and July increased floral bud retention 1.9- and 2.0-fold, respectively. BA (25 mg L^{-1}) plus low-biuret urea applied in June and July or in May, June and July increased floral bud retention 1.6- and 1.7-fold compared to the untreated control. Increasing the BA concentration to 50 mg L^{-1} and applying it in combination with low-biuret urea in May, June and July increased floral bud retention to that obtained with the two algal extract treatments, i.e., 1.9-fold.

Yield

For the yield experiment in Madera, Calif., the on-crop year was followed by an off-crop year. Yield decreased almost 90% from 59 kg fruit (fresh wt) per tree in the on-crop year to only 7 kg fruit (fresh wt) per tree the following year (data not shown). The alternate bearing index (ABI) for the control trees for the 2 years of the study was 0.78 (based on kg fruit [fresh wt] per tree). As anticipated, the treatments significantly affected yield only in the off-crop year, which had a significant positive effect on cumulative yield. All cytokinin and N treatments significantly increased 2-year cumulative yield as kilograms fruit (fresh wt) per tree ($P = 0.0005$) and kilograms split nuts (dry wt) per tree ($P = 0.0018$) (Table 3). Foliar-applied BA combined with low-biuret urea applied in June and July significantly increased the yield (kg/tree) of both fresh fruit (1.8-fold) and split nuts (1.6-fold) compared to untreated control trees. The treatment reduced the alternate bearing index to 0.58 (based on kg fruit [fresh wt] per tree) for the 2 years of the study, the lowest for any treatment (data not shown). Initiating this same treatment one month earlier during fruit set in May significantly reduced the yield of both fresh fruit and split nuts per tree, but both yields were significantly greater than those of control trees. Doubling the amount of BA (138 g ha^{-1}) applied with the low-biuret urea and applying the treatment in May, June and July resulted in intermediate yields of fruit and split nuts that were not significantly different from any other treatments except the control. The algal extract combined with low-biuret urea applied in May, June and July also resulted in a fruit yield that was intermediate to other treatments, but significantly greater then the control. However, this treatment resulted in a yield of split nuts in kg per tree that was equal to that of trees receiving BA plus low-biuret urea in June and July. When the algal extract was combined with N-P-K fertilizer instead of low-biuret urea, yields of fruit and split nuts were slightly lower but still significantly greater than those of the untreated control trees. No treatment had a significant effect on individual nut fresh weight or dry weight, or on percent split nuts, nuts with aborted embryos or nuts with no embryo (blanks) (data not shown).

In the 5-year study, foliar-applied algal extract combined with low-biuret urea had no significant effect on either kg fruit (fresh wt) per tree or kg split nuts (dry wt) per tree in year 1, the on-crop year, although yields were numerically lower than the control. Leaflet tip burn was observed on the treated trees. There was also no significant treatment effect in the following off-crop year (Table 4). The alternate bearing cycle was reset by insufficient chilling during the winter following the harvest of year 2, resulting in a second off-crop year (year 3), which was followed by an on-crop year (year 4). The foliar-applied algal extract plus low-biuret urea treatment had no effect on either kg fruit (fresh wt) per tree or kg split nuts (dry wt) per tree for the second off-crop year. However, in the subsequent on-crop year (year 4), the treatment significantly increased the kg fruit (fresh wt) per tree compared to the untreated control ($P = 0.002$), but this did not translate to a significant increase in kg split nuts (dry wt) tree in year 4 (Table 4). The algal extract plus low-biuret urea treatment had no significant effect on yield in the final year of the experiment, which was an off-crop year (year 5). Overall, the algal extract plus low-biuret urea treatment minimally increased the 5-year cumulative yield as kg fruit (fresh wt) per tree compared to the control at $P = 0.08$, but the effect of the treatment on the 5-year cumulative yield of split nuts (dry wt) per tree was insignificant ($P = 0.12$).

The algal extract plus low-biuret urea treatment had no positive or negative effects on the nut quality parameters evaluated in this experiment (nut fresh weight, dry weight, and percent split nuts, nuts with aborted embryos and nuts with no embryos [blanks]) compared to the untreated control for any year of the study (data nor shown). In addition, no significant effect on nut quality could be attributed to year or the on- or off-crop status of the trees (statistical analyses not shown).

The algal extract plus low-biuret urea treatment had no significant effect on the 2-year cumulative yield for the on-crop years (Table 5). However, the algal extract plus low-biuret urea treatment increased the 3-year cumulative yield for the off-crop years as kg fruit (fresh wt) per tree ($P = 0.004$) and as kg split nuts (dry wt) per tree ($P = 0.10$) (Table 5).

The algal extract plus low-biuret urea treatment had a variable effect on ABI. The treatment significantly reduced the ABI of treated trees compared to the control trees for years 1 to 2 and years 4 to 5 but increased the ABI over that of the control trees for years 3 to 4 (Table 6). Thus, the overall effect of the algal extract plus low-biuret urea treatment on the average degree of alternate bearing across the 5 years of the experiment was insignificant (Table 6).

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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Tables

Table 1. Change in endogenous cytokinin concentrations in floral buds from untreated on-crop 'Kerman' pistachio trees in an orchard in Madera, Calif., during the period of floral bud abscission from the initiation of embryo growth (14 June) to the period of rapid embryo growth (6 July).

	June 14	July 6	*P*-value[z]	Net change (%)
	------- *ng g^{-1} dry wt buds* -------			
Zeatin riboside	182.2	104.0	0.07	–43
Isopentyladenosine	20.2	12.2	0.06	–40

[z] Significant differences between dates determined by student's *t*-test at $\alpha = 0.05$.

Table 2. Effect of foliar-applied cytokinin and N treatments on floral bud retention on shoots of on-crop 'Kerman' pistachio trees in an orchard in Madera, Calif.

Treatment	Date of application	Final bud number (4 Sept.)	Percent floral bud retention (4 Sept.)
Control	–	2.47 b[z]	27 b
BA + urea[y]	12 June + 8 July	4.00 a	43 a
BA + urea	12 May, 12 June + 8 July	4.26 a	44 a
BA (2x) + urea	12 May, 12 June + 8 July	4.63 a	46 a
Algal extract + urea	12 May, 12 June + 8 July	4.69 a	47 a
Algal extract + NPK	12 May, 12 June + 8 July	4.97 a	49 a
P-value		≤ 0.05	≤ 0.05

[z]Values in a vertical column followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$.

[y]BA (6-benzyladenine, MaxCel®, Valent BioSciences Corp.) (25 mg L^{-1}) with low-biuret urea (Unocal PLUS®, Unocal Corp., 20% N, < 0.1% biuret) (0.25% N); BA (2x) (50 mg L^{-1}) with low-biuret urea (0.25% N); algal extract (Binary CQ®, Helena Chemical Co.) (0.08%) with low-biuret urea (0.25% N) or with N-P-K liquid fertilizer (11-8-5, Bayfolan®, Helena Chemical Co.) (0.17%). The application times correspond to fruit set (May), initiation of embryo growth (June) and beginning of the period of rapid embryo growth (July).

Table 3. Effect of cytokinin and N treatments applied annually to the foliage of 'Kerman' pistachio trees in an orchard in Madera, Calif., on 2-year cumulative yield.

Treatment	Date of application	Kg fruit (fresh wt) /tree	Kg split nuts (dry wt) /tree
Control	–	40.30 c[z]	18.35 c
BA + urea[y]	12 June + 8 July	70.73 a	29.27 a
BA + urea	12 May, 12 June + 8 July	56.46 b	23.20 b
BA (2x) + urea	12 May, 12 June + 8 July	62.58 ab	26.77 ab
Algal extract + urea	12 May, 12 June + 8 July	62.58 ab	28.33 a
Algal extract + NPK	12 May, 12 June + 8 July	58.09 b	26.26 ab
P-value		0.0005	0.0018

[z]Values in a vertical column followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$.

[y]BA (6-benzyladenine, MaxCel®, Valent BioSciences Corp.) (69 g ha^{-1}) with low-biuret urea (Unocal PLUS®, Unocal Corp., 20% N, < 0.1% biuret) (6.9 kg ha^{-1} N); BA (2x) (138 g ha^{-1}) with low-biuret urea (6.9 kg ha^{-1} N); and algal extract (Binary CQ®, Helena Chemical Co.) (2.4 L ha^{-1}) with low-biuret urea (6.9 kg ha^{-1} N) or with N-P-K liquid fertilizer (11-8-5, Bayfolan®, Helena Chemical Co.) (4.7 L ha^{-1}) per application in 2804 L ha^{-1} water (adjusted to pH 5.5). The application times correspond to fruit set (May), initiation of embryo growth (June) and at the beginning of the period of rapid embryo growth (July).

Table 4. Effect of algal extract and low biuret urea-N applied in early to mid-June and early to mid-July annually to the foliage of ‘Kerman’ pistachio in an orchard in Kettleman, Calif., on the annual yield and 5-year cumulative yield.

	Yield										5-year cumulative	
	Year 1		Year 2		Year 3		Year 4		Year 5			
	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree	kg fruit (fresh wt) /tree	kg split nuts (dry wt) /tree
Algal extract + urea[z]	41.7	23.9	33.0	12.1	35.5	9.9	67.7 a[y]	22.6	47.3	16.8	224.2	85.4
Control	44.3	29.1	26.3	4.9	37.3	9.7	64.0 b	21.1	39.8	14.0	212.9	78.8
P-value	0.29	0.22	0.21	0.11	0.51	0.77	0.002	0.18	0.16	0.14	0.08	0.12

[z]Algal extract (Binary CQ®, Helena Chemical Co.) at 4.7 L plus low-biuret urea (granular 0.46%, 0.25% biuret) at 28 kg N in 2506 L water per ha per application at the initiation of embryo growth (early to mid-June) and at the beginning of the period of rapid growth (early to mid-July).

[y]Values within a vertical column followed by different letters are significantly different by Duncan’s multiple range test at $P = 0.05$

Table 5. Effect of algal extract and low biuret urea-N applied in early to mid-June and in early to mid-July annually to the foliage of ‘Kerman’ pistachio in an orchard in Kettleman, Calif., on the cumulative yield for the two on-crop years (years 1 and 4) and for the three off-crop years (years 2, 3 and 5).

	On-crop 2-year cumulative yield		Off-crop 3-year cumulative yield	
	kg fruit (fresh wt)/tree	kg split nuts (dry wt)/tree	kg fruit (fresh wt)/tree	kg split nuts (dry wt)/tree
Algal extract + urea[z]	109.6	46.5	115.1 a	38.9
Control	108.2	50.2	102.9 b	28.6
P-value	0.66	0.31	0.004	0.10

[z]Algal extract (Binary CQ®, Helena Chemical Co.) at 4.4 L plus low-biuret urea (granular 0.46%, 0.25% biuret) at 28 kg N in 2506 L water per ha per application at the initiation of embryo growth (early to mid-June) and at the beginning of the period of rapid embryo growth (early to mid-July).

[y]Values within a vertical column followed by different letters are significantly different by Duncan’s multiple range test at $P = 0.05$.

Table 6. Effect of algal extract and low biuret urea-N applied in early to mid-June and again in early to mid-July annually to the foliage of 'Kerman' pistachio in an orchard in Kettleman, Calif., on the alternate bearing index for the five crop years based on kg fruit (fresh wt) per tree.

	Years				5-year average
	1-2	2-3	3-4	4-5	
Algal extract + urea[z]	0.41	0.46	0.36	0.20	0.36
Control	0.50	0.49	0.28	0.29	0.39
P-value	0.0196	0.5264	0.0474	0.0002	0.2772

[z]Algal extract (Binary CQ®, Helena Chemical Co.) at 4.7 L plus low-biuret urea (granular 0.46%, 0.25% biuret) at 28 kg N in 2506 L water per ha per application at the initiation of embryo growth (early to mid-June) and at the beginning of the period of rapid embryo growth (early to mid-July).
[y]Values within a vertical column followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$.