PISTILLATE FLOWER ABORTION AND ETHYLENE PRODUCTION IN WALNUT

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ABSTRACT

This year's research focused on further evaluation of potential alternate bearing with multiple year ReTain™ treatments. After three years of treatment with ReTain™, there does not appear to be any negative effect on yield. There was a lower reduction in PFA than was expected, but this is thought to be unrelated to multiple year treatments. Treatment of a Chandler orchard with ReTain™ was done to evaluate the potential to reduce PFA, particularly close to pollinizers. There was no reduction in PFA in this orchard. This result is the same as seen last year, at a different location. The inhibitor of ethylene reception, 1-MCP, was tested for the first time on whole tree applications. 1-MCP significantly reduced PFA, but no differences in individual tree yields were found. In vitro ethylene measurements further indicated that a peak in ethylene production occurs after pollination. This study also indicated that both the stigma and ovary produce ethylene, although the stigma appears to produce more. These flowers will be used for RNA extraction and for analysis of ethylene-related gene expression with time from pollination. An effective protocol for isolation of small quantities of walnut RNA has been developed. Further evaluation of historical climate and bloom data indicates that there are distinctive chilling requirements for pistillate and staminate bloom. Further refinement is necessary for development of an accurate predictive model.

OBJECTIVES

Evaluate any influences of ReTain™ on alternate bearing in a Serr orchard treated for three years.

Conduct a whole tree trial on 1-MCP in a Serr orchard to determine effectiveness in reducing PFA.

Conduct a ReTain™ trial in a Chandler orchard to determine if there is a significant reduction in PFA, particularly close to pollinizers.

Conduct laboratory experiments to further evaluate in vitro ethylene production.

Extract RNA to evaluate gene expression with pollination that might affect ethylene production.

Evaluate historical climate and bloom data to determine if differential chilling and/or post chilling heat unit accumulation requirements exist for staminate and pistillate bloom for Serr and Chandler cultivars.

PROCEDURES

Alternate bearing with ReTain™ treatment

The San Joaquin County trials were conducted in a mature (planted 1973) 27-acre Serr orchard with a closed in canopy roughly 45 to 50 feet tall. The test orchard had single rows of cv. Tehama pollinizers planted every 10 rows of Serr trees and was adjacent to a 30-acre block of nonbearing Hartleys on the west and 20-acre block of mature Paynes on the east. There were no walnuts immediately north and south of the test orchard.

ReTain was applied at 125 ppm; controls were untreated. Treatments were applied in 200 gallons of water per acre using a commercial air blast sprayer. No spray adjuvants were added to experimental treatments. Other than the experimental treatments, cultural and pest management practices were similar and considered standard for Serr in the northern San Joaquin Valley.

In this block 2004 and 2005 treatments resulted in high-yielding trees from the ReTain treatments where PFA was reduced, and low-yielding trees from the controls where PFA was high. Half of the high-yielding trees and half of the low-yielding trees were sprayed with 125ppm ReTain. The others were left as untreated controls.

The day after treatments were applied (April 20), fifteen shoots having two open pistillate flowers were tagged for future set evaluation on two center trees of each plot. The numbers of nuts on tagged shoots were counted on May 9, and again on May 26.

Harvest was performed twice (October 3 and 10) on each experimental six-tree plot. During each harvest, an approximately 20 pound random sample was collected from the harvest cart and used to calculate a dry, in-shell conversion factor following commercial hulling and drying. A five-pound sub-sample was collected from second shake samples and submitted to Diamond Foods, Inc. for third party nut quality evaluation.

SmartFresh (1-MCP) Serr whole tree trial

The 1-MCP trial was conducted in a mature Serr orchard in northern San Joaquin County. Treatments were applied in a Latin square design with single tree replicates, and four trees of each treatment. Treatments consisted of SmartFresh at: 50 g ai/ha, 100 g ai/ha, a water control, and an untreated control. Treatments were applied using a hand gun sprayer and 350 gal/A. Treatments were applied and flower pairs tagged on April 13. The numbers of nuts on tagged shoots were counted on May 2, and again on May 16. Individual tree harvests were conducted on September 19. Approximately 20 lb. random samples were taken from each tree and used to calculate a dry, in-shell conversion factor. Samples were submitted to Diamond Foods, Inc. for third party nut quality evaluation.

ReTain 'Chandler' Trial

This experiment was conducted in a San Joaquin County 'Chandler' orchard with a history of PFA. Cisco pollinizers are planted every fourth row along the north and west sides of the orchard.

Plots consisted of eight trees on a transect with increasing distance from Cisco pollinizers. Four replications of each treatment were used. Plots were randomly assigned a treatment of untreated, water control or 125 ppm ReTain. Treatments were applied using the grower's 'speed' sprayer. On May 4, flowers were tagged. The numbers of nuts on tagged shoots were counted on May 25 and on June 14. The first count was delayed due to rain.

In Vitro Ethylene Synthesis

One to two foot long walnut shoots from Serr trees were collected shortly after budbreak. Shoot were re-cut in the lab under water, and placed in jars containing 5% bleach solution. As flowers became receptive, they were pollinated, and tagged. A given time after pollination, flowers were removed from the shoot, placed in small vials and sealed. Gas samples from the vials were taken, and ethylene concentrations were determined using standard gas chromatography protocols. Unpollinated controls were also used. Some flowers were divided into stigma and ovary portions for measurements of ethylene evolution.

RNA extraction and gene expression

Standard methods for the extraction of RNA were tested to determine the most efficient for small-scale RNA extraction from walnut tissue. Trizol, CTAB, and hot borate were all tested, along with modifications of them. A hot borate method, modified for small amounts of tissue was developed and determined to be the most effective. Due to limited public walnut DNA resources, tests to determine usable primers for amplification of cDNA are being conducted.

Evaluation of historical bloom and climate data

Continued evaluation of historical bloom and climate data. Data from the walnut improvement program, as well as local weather data were used in a statistical method of determining chill units and growing degree hour requirements (Ashcroft *et al.,* 1977). An estimate of chilling requirement was made from evaluating hours below 45 F, and heat units to bloom. From this baseline, the end of rest date (chilling completed date) was calculated, and growing degree hours (GDH) were calculated from then until bloom. This was repeated for six years of data. The standard deviation of the GDH was calculated. These steps were repeated from the original estimate and increments of 25 chilling hours from it. The CU requirement with the lowest standard deviation in GDH (least variation) was selected. Average GDH for that number of chilling units was calculated. The estimated CU and GDH was evaluated by using to calculate bloom dates for years that were not used to develop the model.

RESULTS AND DISCUSSION

Alternate bearing with ReTain™ treatment

After applying ReTain™ treatments for three years, no significant differences in yields were seen, but yields were significantly higher in all treated plots than in the untreated ones (figure 1). Yields were low, even in the treated plots. This is due, in part to a lower than expected decrease in PFA with ReTain™ treatment (figure 2). Although the drop was less with ReTain™ treatment, we have previously seen a much greater reduction in drop. The causes of this are unclear, but it is unlikely to be related to multi-year treatments. A similar low level reduction in PFA was seen in a section of this orchard that had never been treated. At least at these low levels of production, there appears to be no effect of previous year's treatment on yield.

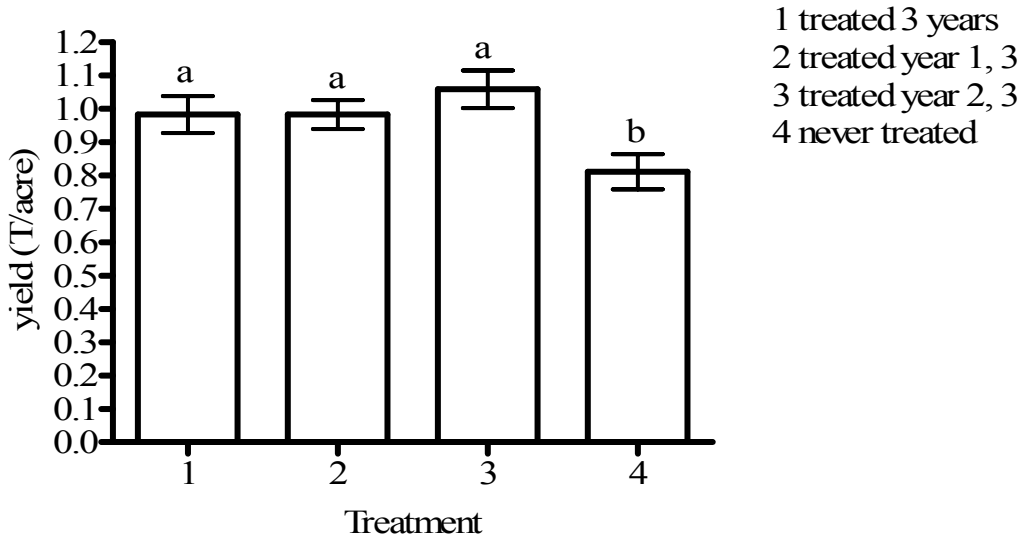

Figure 1. Yield of Serr trees treated with ReTain for multiple years.
Letters indicate significant differences between treatments (p≤05).

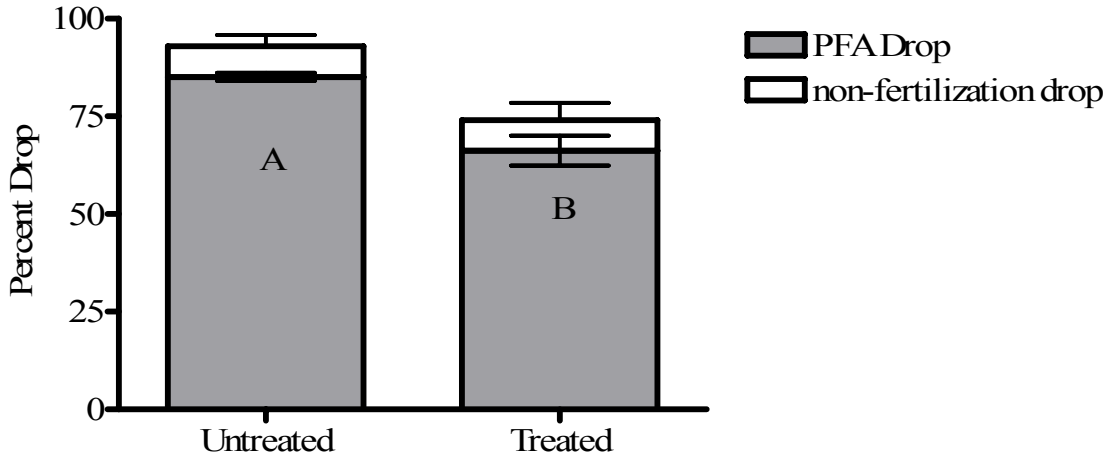

Figure 2. Serr flower drop in treated (125 ppm ReTain), and untreated controls. Letters indicate significant differences in PFA drop (p≤05). No differences in non-fertilization drop were detected.

SmartFresh (1-MCP) Serr whole tree trial

Whole tree applications of 1-MCP reduced PFA drop. There was a dose response seen with the two different concentration of 1-MCP used, with the 100 g ai/ha rate significantly reducing PFA below that of the controls (figure 3). Further large scale studies should be conducted to determine what the optimum rate is. Although there was a trend of higher yields with treatment, no differences in yield between any treatment were seen (figure 4), most likely due to variation in tree size, and individual trees being used as replicates. Previous research indicates that with a 30% reduction in PFA, yield differences should be seen. A ReTain™ trial by Joe Grant in a different part of this same orchard showed a similar 30% reduction in PFA, but had the expected corresponding increase in yield.

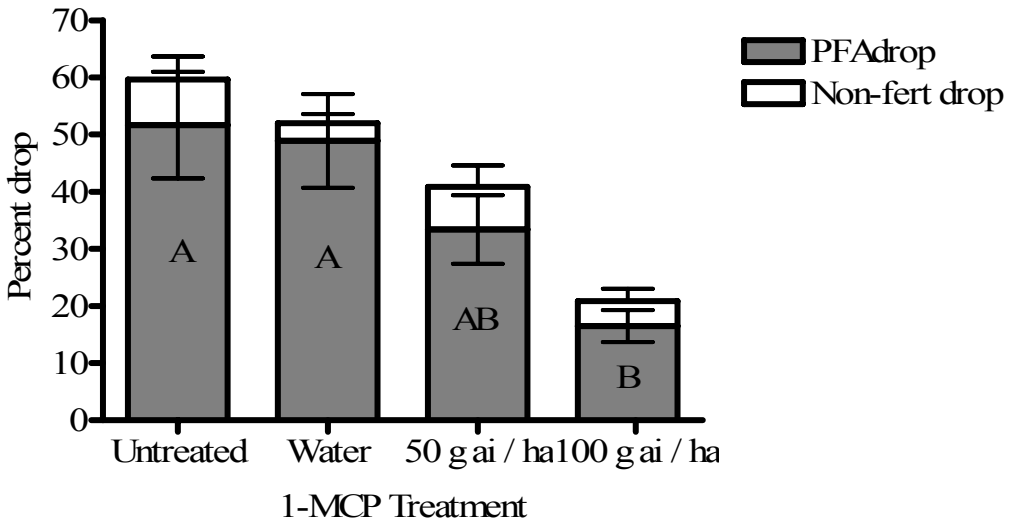

Figure 3. Letters indicate significant differences in PFA drop ($p \leq 05$). No significant differences in the non-fertilization drop were detected.

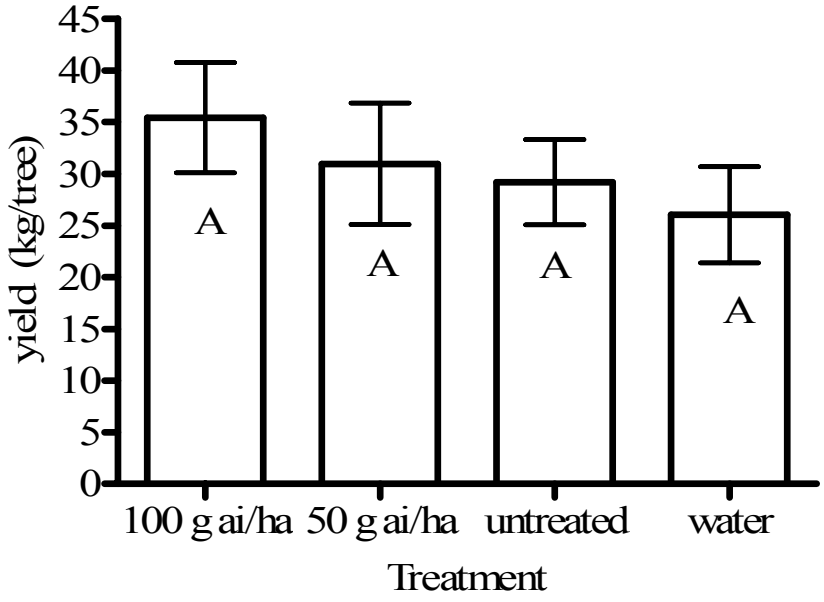

Figure 4. No significant differences in yield were detected between treatments ($p \leq 0.05$).

ReTain™ 'Chandler' Trial

No significant reductions in PFA with ReTain™ were seen at any distance from the pollinizer. This is the second study using ReTain™ on Chandler trees (see 2005 report), where there has been no reduction in PFA. Although we have seen reduction in PFA below these levels (of approximately 25%), there does not appear to be the same control in the Chandler trees as in Serr.

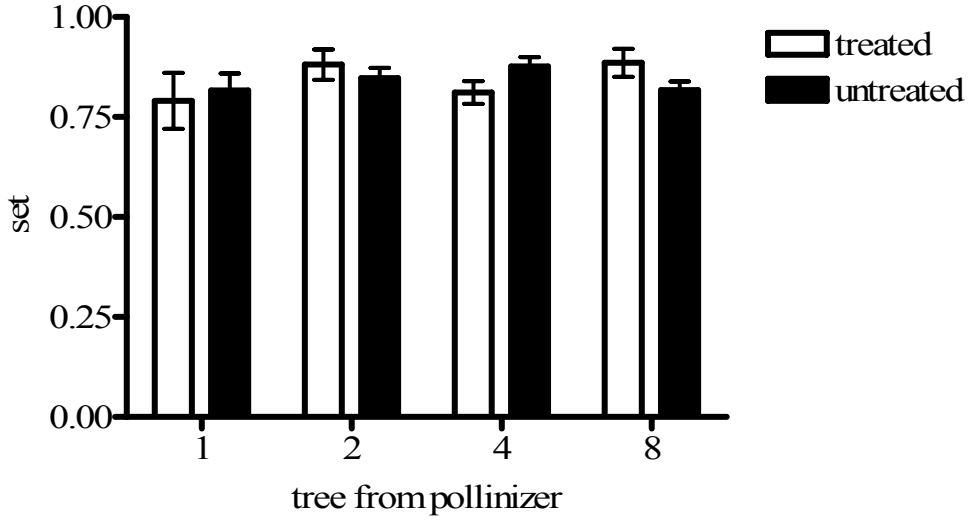

Figure 5. Fruit set in Chandler trees. Trees were treated with ReTain at 125 ppm, or left untreated. No significant differences were detected.

In Vitro Ethylene Synthesis

Ethylene production in pollinated flowers remained low immediately after pollination, but did eventually increase, and peaked approximately 20 hours after pollination (figure 6). Some flowers may have had a later peak, causing the low levels of ethylene production in some of the measurements. When ethylene production was measured separately for the stigma and the ovary, the stigma appears to produce more ethylene than the ovary, but both the stigma and the ovule produced large amounts of ethylene compared to the unpollinated flowers (figure 7).

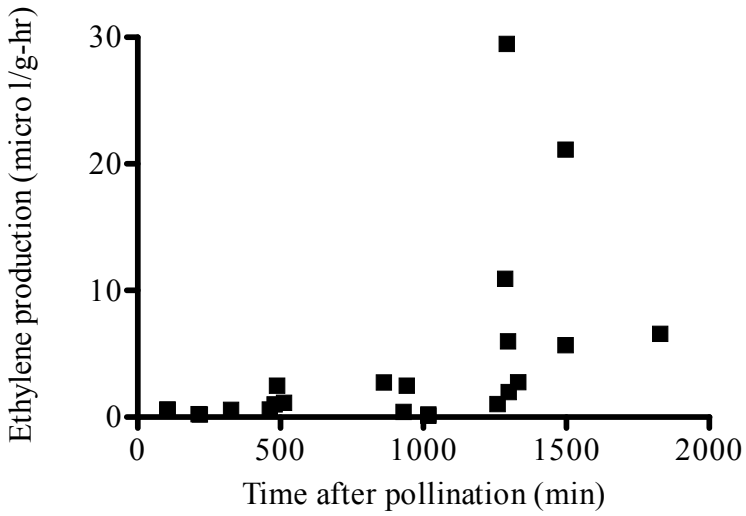

Figure 6. In vitro ethylene production by pollinated walnut flowers.

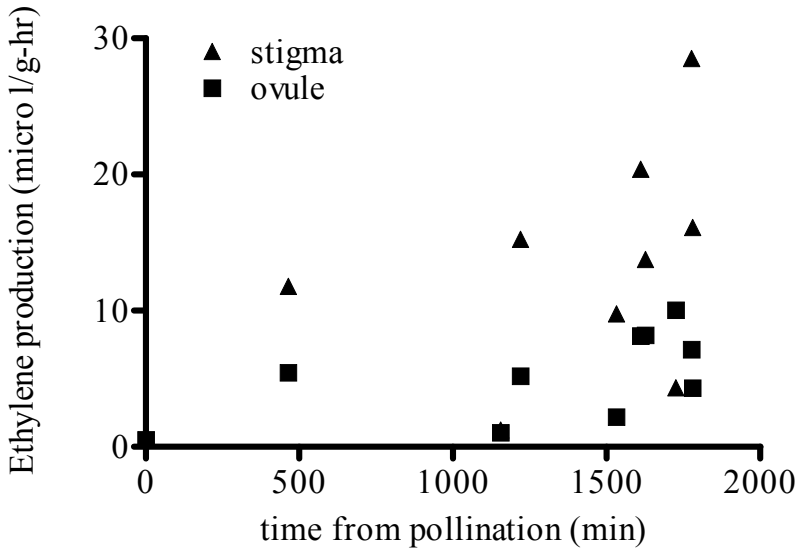

Figure 7. Ethylene production by stigmas and ovary of pollinated walnut flowers.

RNA extraction and gene expression

A new methodology for extracting walnut RNA has been developed. The next steps will be to finish evaluating primers for ethylene related genes, and to evaluate gene expression based on time after pollination.

Evaluation of historical bloom and climate data

Continued evaluation of historical bloom data further indicates a differential chilling requirement for pistillate and staminate flowers. Minimum standard deviation (SD) occurs at 550-575 chilling hours for pistillate bloom, and 700 chilling hours for staminate bloom. This differential chilling requirement may contribute to yearly variation in bloom overlap and PFA. Differences in GDH probably exist, but are minor. The results of testing the model on years that were not included in its development were variable. Further testing and development of the model are needed for more accurate prediction of bloom time and overlap.

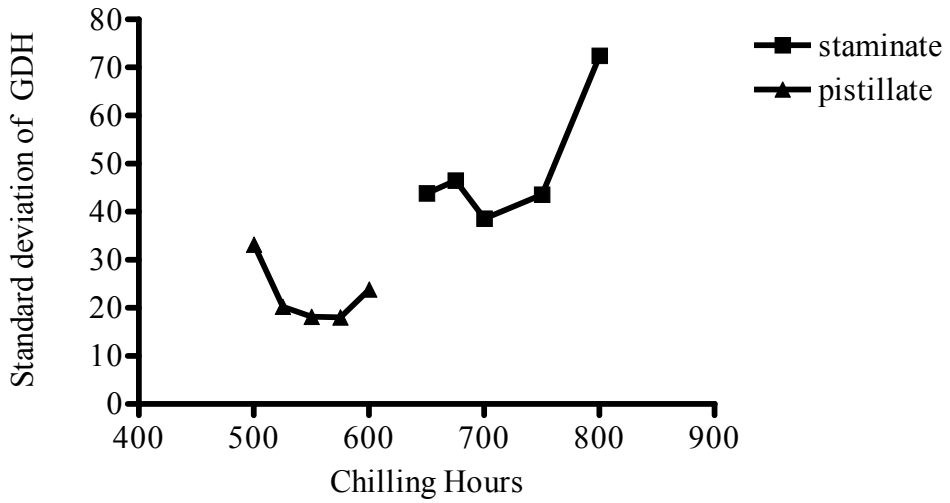

Figure 8. Standard deviations of GDH (from estimated chilling completion to first bloom) for estimates of chill unit requirements for staminate and pistillate bloom.