WALNUT POLLINATION AND PISTILLATE FLOWER ABORTION

V. Polito, J. Grant, and H. Johnson

ABSTRACT

We have been investigation the physiology and efficacy of ethylene antagonists as management tools for controlling PFA in walnut orchards, especially in Serr orchards. We focused primarily on aminoethoxyvinylglycine hydrochloride (AVG) as ReTain (Valent Biosciences) as a management tool for controlling PFA and have begun research on a sprayable formulation of 1-methylcycloproane (1-MCP), a product of AgroFresh, Inc. The modes of action for these two inhibitors are distinct. AVG interferes with ethylene synthesis. 1-MCP is a competitive inhibitor of ethylene action. In this report we show that AVC does reduce ethylene synthesis in pollinated walnut flowers. By contrast, 1-MCP appears to increase ethylene synthesis, possibly due to feedback mechanisms triggered by the effect of the chemical in blocking ethylene receptors. Previous work by Beede and Grant showed that ReTain can reduce PFA in field trials. Grant repeated this trial in the San Joaquin county 'Serr' orchard this year; results were consistent with previous results showing the efficacy of AVG in controlling PFA. In a second field trial, Johnson treated a Serr block in San Joaquin county with a second year AVG (ReTain) treatment. Results show no statistical differences between trees that were high yielding (AVG-treated) the previous year and those that were low yielding (controls) the previous year for this year. In this experiment, AVG-treated (125ppm) trees showed a 57 to 70% yield increase over the untreated controls. A field experiment by Johnson in a 'Chandler' orchard was inconclusive due to other yield-limiting factors in that orchard in addition to PFA. In a preliminary, branch-level study with 1-MCP on 'Chandler' there was good control of PFA in 3 and 10 ppm applications, especially for prereceptive and postreceptive pistillate flowers.

OBJECTIVES

Repeat previously reported experimental protocol of 2004 to validate or refine use of ReTain (aminovinylglycine hydrochloride, Valent BioSciences Corporation) to reduce PFA in Serr walnut. Repeat application of ReTain for a second year in a previously treated block to determine if there are any carryover effects of successive year treatments.

Conduct a field trial of ReTain in a 'Chandler' orchard to determine if it results in a reduction of PFA, particularly in trees close to pollinzers.

Conduct preliminary field trials of 1-methylcyclopropane (1-MCP, AgroFresh, Inc.) to determine if it is effective in reducing PFA.

Characterize in vitro, the responses of AVG and 1-MCP on ethylene- production by pollinated walnut flowers.

PROCEDURES

Northern San Joaquin Valley Field Trials.

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 pollenizers 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. Two plots were used. One (Trial 1) was a different area of the same orchard from that used for a ReTain efficacy trial in 2004. The other (Trial 2) was a trial repeated in the same block used in 2004.

Trial 1.
Four treatments were assigned to four six-tree plots using a completely randomized block design with blocks arranged north to south in two pairs of rows separated by a single untreated row in the orchard. Plots were separated by at least one untreated tree within rows. Test rows were separated from Tehama pollenizer by three rows of Serr on the west and one row on the east. Prevailing winds during Serr and Tehama pollen shedding were from both the northwest and southeast, providing for good pollen movement across rows.

Experimental treatments were: 1) ReTain at 125 ppm, 2) ReTain at 62.5 ppm, 3) water only, and 4) untreated. All 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. ReTain and water applications were made at 80-90% Serr pistillate bloom on March 31 between 8 and 10 am.

The day treatments were applied, fifteen shoots having two open pistillate flowers were tagged for future set evaluation on two center trees of each plot. The number of nuts on tagged shoots were counted on April 19 and again on May 19.

Harvest was performed twice (September 15 and 27) on each experimental six-tree plot. During each harvest, a 25-35 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.

Treatment separation for San Joaquin County data was determined using Fisher's Protected LSD test at $p=0.05$.

Trial 2.
ReTain was applied at 125 ppm; controls were treated with water only. 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 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 sprayed with water only as controls.

Return bloom was evaluated for the 2004 high-yielding (ReTain treated) and low yielding (controls) trees. Flowers were tagged. PFA and post-PFA flower drop was recorded and harvests were done as above.

ReTain 'Chandler' Trial

This experiment was conducted in a Solano County 'Chandler' orchard with a history of poor yields. The orchard has approximately 5% 'Franquette' pollinizers which are planted every 20th row. The orchard was selected for this trial because it was considered that PFA may have been a factor limiting yield.

Plots consisted of ten trees (a Franquette pollenizer, and 9 Chandler trees on a transect with increasing distance from the Franquette). 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 two days after first bloom. Five days after treatment, pairs of receptive flowers were tagged on Chandler trees 1, 2, 4 and 8 trees from a 'Franquette' pollinizer. Two weeks later, the number of flowers remaining was recorded and final set was recorded in July.

1-MCP Branch Study.

Branches were sprayed with 1-MCP in a isopropanol-based formulation at 1, 3 and 10 ppm. All flowers were tagged and scored according to stage at time of treatment: Pre-receptive, receptive, post-receptive. Tagged flowers were scored for drop after two weeks and in mid July.

In Vitro Ethylene Synthesis

Walnut pistillate flower pairs were collected at early receptive stages. They were placed in vials in sets of three and held in the lab for 24 hours. After 24 hours treated flowers were pollinated with freshly collected walnut pollen. Controls were left unpollinated. The vials were placed in sealed jars. At intervals following pollination, head space samples were taken from the jars and ethylene concentrations determined using standard gas chromatography protocols.

RESULTS AND DISCUSSION

Northern San Joaquin Valley Field Trials.

Trial 1.
Observations of staminate (catkins) and pistillate (female flowers) bloom of Serr and Tehama pollenizer trees in the test orchard indicated that pollen shedding by Serr was nearly complete by the time Serr pistillate bloom began. Tehama pollenizers shed pollen throughout nearly the entire Serr pistillate bloom period.

The first set counts performed April 19 were timed to assess loss of flowers due to PFA and the May 19 counts to assess losses from both PFA and other causes of drop; the latter is thought to be primarily due to a lack of effective pollination. Previous work has shown that PFA is typically concluded within two to three weeks after bloom. Flowers unaffected by PFA continue to grow, and a small percentage (e.g. 3-5% in 2004) abscise within the following few weeks due to ineffective pollination. This "non-pollination" drop was abnormally large this year, accounting for around half of the observed reduction in final nut set (Table 1). We believe this result is an artifact of the timing of our first set count and that a much greater portion of the overall drop than is apparent was due to PFA, not ineffective pollination. We have not previously observed non-pollination drop levels greater than around 8 to 10%. For unknown reasons, we speculate that PFA occurred over a longer period than normal, that our first set counts were made before PFA had ended, and the second count thus over-estimates the portion of drop due to ineffective pollination. If this interpretation is correct, a large portion of the significant reductions in non-pollination and overall drop from ReTain treated trees were attributable to PFA.

Treating with 125 or 62.5 ppm ReTain significantly increased first shake yields (Table 2) compared to water-treated and untreated trees. Second shake yield was significantly greater from trees with 125 ppm ReTain than all other experimental treatments. Total (1^{st} plus 2^{nd} shake) yield was greatest from trees treated with 125 ppm ReTain, intermediate from 62.5 ppm-treated trees and lowest from water-treated and untreated trees. Compared to untreated, ReTain treatment at 125 ppm increased average yield by 47%; at 62.5, yield was increased 28%.

There were no significant differences among treatments in second-shake nut quality parameters we evaluated, except for a small increase in the percentage of "large sound" nuts from trees treated with ReTain at 62.5 ppm (Table 3).

Trial 2.
For this trial, a second year treatment of ReTain was applied on 'Serr'.

Return bloom—determined as percent floral shoots and number of flowers per floral shoot (Fig. 1)—did not differ in the high and low yielding trees.

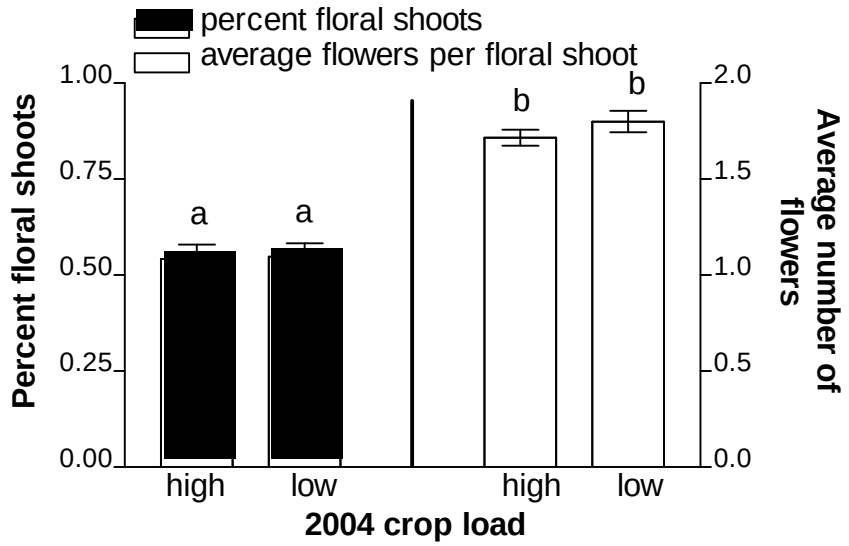

Figure 1. Return bloom in 2005 for ReTain treated (high yielding) and Control (low-yielding) trees from the 2004 experiments.

PFA and post-PFA drop was determined for the 2004 high and low yielding trees, each of which received a 125ppm ReTain treatment and a water control treatment. The ReTain treatment significantly reduced PFA with no effect on post-PFA drop in either the previous year's high or low yielding trees (Fig. 2).

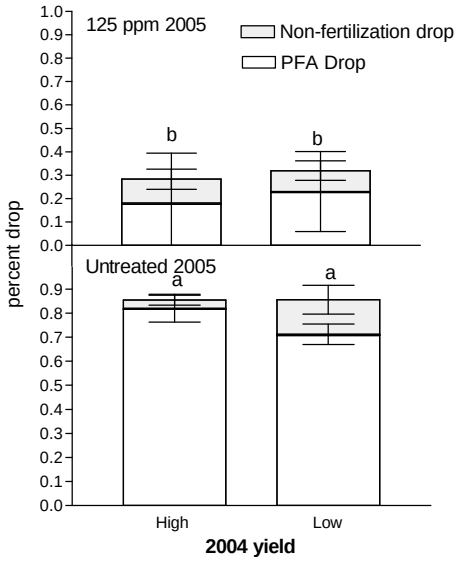

Figure 2. PFA for 2005 following ReTain (125ppm) and water controls treatments of trees that were high-yielding (2004 ReTain treated) and low yielding (2004 controls) the previous year.

Yields were significantly increased in the 2005 ReTain treated trees regardless of 2004 treatment and associated yield status (Fig. 3). There is some indication (although it is not significant) of an alternate bearing effect between the 2004 high and low yielding trees in both the 2005 ReTain treatment and the water control. For each case, there was an approximately 1/3 ton/acre yield

differential that may be attributable to alternate bearing. This result suggests that a third year experiment on this same set of trees will provide valuable information to help clarify this phenomenon, especially with the lack of statistical significance evident in this observation. For the 2004 low-yielding trees the ReTain treatment increased yield over water controls by 70%. For the 2004 high-yielding trees ReTain treatment increased yield over water controls by 57%.

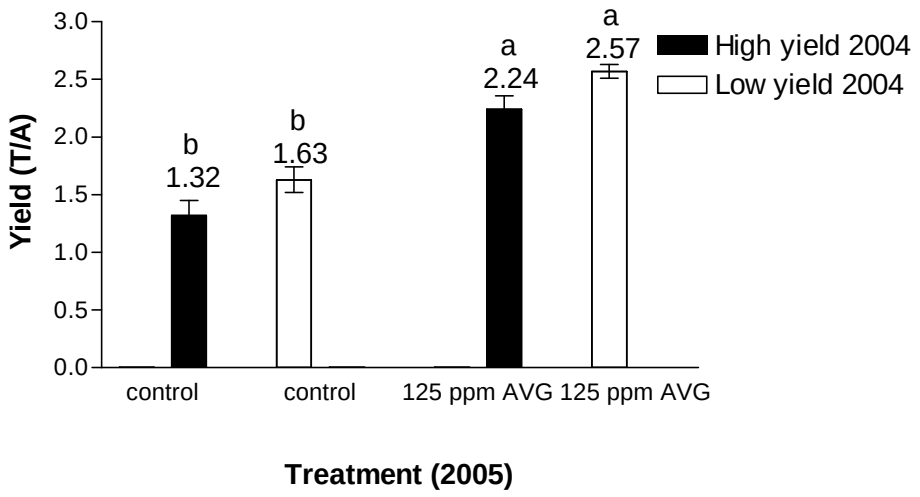

Figure 3. 2005 yields for ReTain treatments and water controls on Serr trees that were high yielding (2004 ReTain treated) and low yielding (2004 controls) the previous year.

Solano County 'Chandler' Trial.

In the ReTain experiment we found no statistical differences in PFA for the125 ppm AVG treatment compared to either the water spray or the untreated controls. We chose this orchard because we felt that PFA was a yield-limiting problem, however PFA was much lower in this orchard than our preliminary assessment suggested it would be. This was the first field trial of AVG on 'Chandler'. It would be premature to take these results to indicate that controlling PFA with ethylene antagonists has no value for 'Chandler' production. We have proposed a follow up experiment in another orchard with the same objective: To determine if ethylene antagonists have an effect on PFA in Chandlers, particularly in trees close to pollinizer varieties.

The branch study using 1-MCP showed a reduction in PFA for pre-receptive and post-receptive pistillate flowers treated with 3 and 10 ppm 1-MCP (Fig. 4). Receptive stage flowers showed lower levels of PFA in the untreated controls. This may be correlated with the presence of pollen in the orchard at the three floral stages.

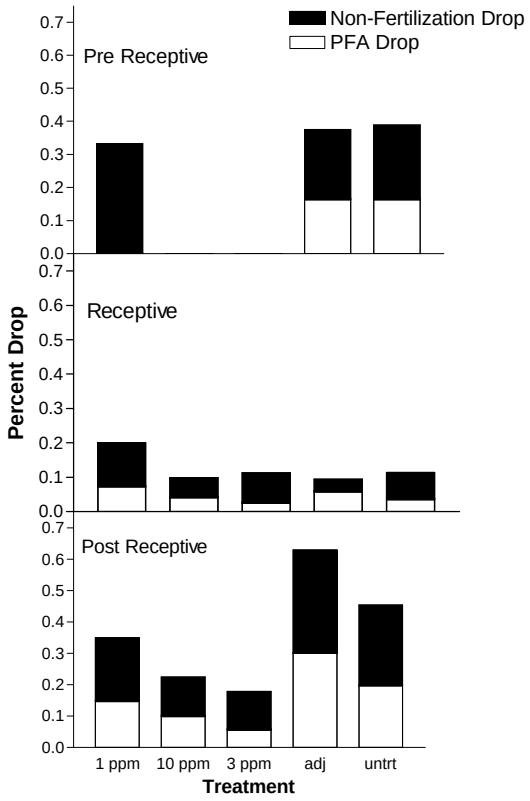

Figure 4. PFA and Post-PFA drop following treatment with 1-MCP at 1, 3 and 10ppm. Controls were the isopropanol-based adjuvant only and no treatment.

These results support additional, large-scale field experiments with 1-MCP. Because 1-MCP is a gaseous compound, it must be applied in a sprayable formulation with an appropriate adjuvant. The formulation must retain the gas until it is released to the plant tissue. AgroFresh has on-going research on development of spray formulations. We propose continuing this work as AgroFresh's research progresses in this area.

Ethylene Evolution in Vitro

Markedly different results were obtained from our in vitro experiments with AVG and 1-MCP. AVG suppressed ethylene production at 50, 100 and 200 ppm compared to control treatments with water sprays, pollinated and unpollinated flowers (Fig. 4). No dose response was seen in these treatments: ethylene levels were not different for any of these concentrations.

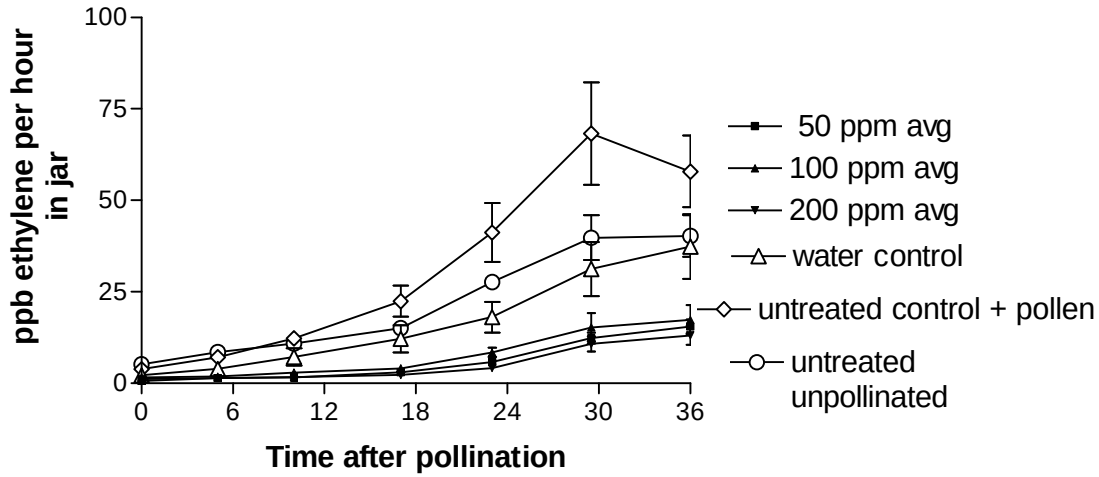

Figure 5. Ethylene evolution from walnut flowers treated with 50, 100 and 200 ppm ReTain, water, untreated pollinated and untreated unpollinated.

For 1-MCP, the chemical was applied in the isopropanol-based adjuvant at 1, 3 and 10 ppm and directly as a gas generated by 2ml of a 3 ppm 1-MCP solution in the vial for 65 min. Here, a clear dose response is seen with increasing ethylene correlated with the 1-MCP spray concentration, and a still greater response from the gaseous form (Fig. 6). These results must be interpreted cautiously, however, as they are preliminary and have not been repeated. We plan to continue these experiments next year.

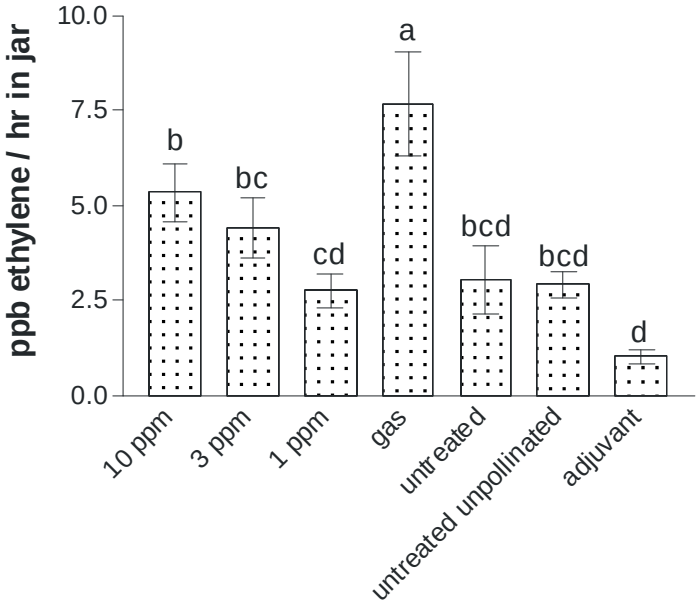

Figure 6. Ethylene evolution from walnut pistillate flowers treated with 1-MCP in isopropanol adjuvant and 1-MCP gas.