POLLEN, PISTILLATE FLOWER ABORTION/ABSCISSION

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ABSTRACT

We have been investigating the role of pollen, especially high pollen levels, as the cause of walnut pistillate flower abortion and abscission (PFA). Our research efforts initially focused on ‘Serr’, the cultivar most strongly affected by PFA. Results presented in previous reports showed that PFA in ‘Serr’ was strongly correlated with high levels of pollen on the pistillate flowers. Dose response curves indicated that pollen doses in the range of 70 to 100 pollen grains per pistillate flower would induce 50% abortion in the pistillate flowers; higher levels of pollen resulted in greater losses. Experimental manipulation of pollen levels in orchard situations by removing pollinizer trees or shaking trees to remove catkins showed that PFA in ‘Serr’ can be reduced by reducing pollen. As self pollen induces PFA just as pollen from pollinizer trees, greater reductions in PFA could achieved by removing catkins from ‘Serr’ trees as well as the pollinizer trees in the orchard. This was most effectively done by shaking the trees at about the time catkin expansion was underway. When trees were shaken as the first catkins began to shed pollen, we could achieve up to 80% removal of catkins. We are currently investigating the possibility of removing catkins by chemical means, but this research is still in the preliminary stages. We do not expect to have useful results in this area for at least two more years.

Our recent work has focused on other walnut cultivars. We found that there is a differential sensitivity to pollen-induced PFA among cultivars. ‘Serr’ is most sensitive; PFA losses in ‘Serr’ could exceed 90% when high pollen levels are experienced. Although no cultivar showed as great a degree of PFA as did ‘Serr’, other cultivars do show similar responses: high pollen loads induce high levels of PFA, and PFA is reduced as pollen load decreases. Of the cultivars we examined in detail, ‘Chico’ showed the lowest levels of pollen-induced PFA, and ‘Chandler’ and ‘Vina’ were intermediate. Results vary among orchards and from year to year, but for ‘Chico’, maximum PFA for trees adjacent to pollinizer rows was approximately 20-25%. ‘Chandler’ and ‘Vina’ PFA typically exceeded 40% in trees adjacent to pollinizers. For each of these, PFA decreased with distance from the pollinizer rows with mean levels of PFA near 10% for ‘Chico’ and 15-20% for ‘Chandler’ and ‘Vina’.

For the past three years we have concentrated primarily on ‘Chandler’ because of its increasingly important role in the walnut industry. A primary concern was to determine if we could identify levels of pollinizers in the orchard that would provide satisfactory amounts of pollen to set a crop, but not so much pollen as to induce unacceptable levels of PFA. Research was conducted in several orchards in various walnut-growing areas around the state. Results were highly variable. Results from the Tulare county indicated that ‘Chandler’ orchards without pollinizers did have somewhat reduced set from lack of pollination, but that greater losses to PFA occurred in the orchards with pollinizers. We also found that removing pollen from ‘Franquette’ pollinizers was correlated with decreased PFA and increased yield; no negative effects from
reducing pollen were apparent. This contrasts with results from other sites. Our work in a San Joaquin county site showed no significant effect of pollen removal and no correlation between PFA and distance from untreated pollen sources. In a Yolo county orchard we found no relationship between distance from pollinizer trees and PFA, but our analysis of drop attributable to insufficient pollination indicated that the presence of pollinizers did reduce losses from unpollinated flowers. Results from three Sacramento valley orchards in Sutter and Butte counties provided no clear indications that PFA was significantly reduced by removing catkins from the pollinizer trees or with distance from the pollinizer trees. However, yield data from the Butte county orchard did show small increases in crop yield with increasing distance from pollen sources. This may indicate that our analyses are not sufficiently detailed to provide a complete picture of PFA levels throughout the entire orchard canopy, and, as a consequence, we may be underestimating somewhat the effects of pollen on PFA in ‘Chandler’ orchards.

Thus, it is difficult to come up with summary generalizations from these results with ‘Chandler’ although there does appear to be an increasing need for pollinizers as one move north through the state. In addition, the need for pollinizers in the orchard is likely to be greater in areas where few other walnut orchards are present nearby. We found significant, but small, losses from lack of pollen in orchards without pollinizers in the southern San Joaquin Valley; but a much greater degree of flower drop due to lack of pollination in the northern regions of the state. We are unable to explain these differences at this time.

Growers should consider the role of pollen-induced PFA in orchard planning. Suggestions of several years ago that ‘Chandler’ walnut orchards should include as much as 10% pollinizer trees now appear to be excessive. However, results do indicate that a small number of pollinizers, perhaps 2 to 3%, are desirable in most orchard situations. Growers must carefully consider their individual situations in making these decisions. In areas where few other walnuts are present and the levels of walnut pollen in the surrounding region can be expected to be low, one might want to increase that number somewhat. Similarly, in areas where there are many walnut orchards contributing high levels of pollen to the ambient environment, one might want to consider using fewer pollinizers. With regard to ‘Serr’, our results indicate that it can be successfully grown, particularly in the southern San Joaquin Valley areas, when it is planted without pollinizer cultivars and efforts are made to reduce pollen load by shaking catkins from the ‘Serr’ trees before they bloom fully.

BACKGROUND

Pistillate Flower Abortion/Abscission (PFA), the loss of pistillate flowers early in the developmental process and prior to fruit set, has been determined to be related to excess pollen load, especially in Serr where the problem is most severe. Earlier results have shown that PFA occurs, although to a lesser extent, in other cultivars.

The PFA phenomenon raises questions regarding the role of pollen and pollinizer cultivars in walnut orchards. This has become a more important issue in cultivars other than Serr, especially Chandler, where reducing pollen sufficiently to reduce PFA may adversely affect the levels of
pollen necessary to maintain normal fruit set. Our results from 1994 in the southern San Joaquin Valley Chandler orchards indicated that PFA, induced by excess pollen when pollinizers are used, may be responsible for more losses than lack of pollination from insufficient pollination in the absence of pollinizers. Last year’s results from the southern San Joaquin area confirmed that conclusion, but data from the northern San Joaquin and Sacramento valley areas were less clear. Anecdotal reports suggest that pollination requirements for Chandler vary in different walnut growing regions and that there may be a greater need for pollen and, therefore, increased numbers of pollinizers in other areas, especially the Sacramento Valley. Therefore, this year we concentrated our efforts on Chandler orchards in San Joaquin, Yuba and Butte counties.

Previous work on Serr PFA has shown a clear benefit from removing pollen sources in Serr orchards. PFA can be greatly reduced, and yield greatly increased by reducing pollen both from pollinizer trees present in the orchard as well as from Serr itself. In general, this has been accomplished by removing pollinizer trees and by shaking Serr trees at a stage appropriate for catkin removal. We have been experimenting with the use of Ethrel sprays to remove catkins. Previous results, conducted on individual branches, showed that catkin abscission was induced by Ethrel sprays in the fall. However, leafing and bloom was delayed, and nut size was reduced. This year we extended those experiments to whole trees.

An additional mode of flower/fruitlet drop has been identified in Chandler. The drop occurs later than that typical of PFA, but earlier than what is typically associated with lack of sufficient pollen. Previous investigations have indicated that PFA or pollination did not appear to be a factor in this problem. This drop has been referred to as "bull" Chandler, "non-productive" Chandler or "off-type" Chandler. The problem occurs intermittently: in some years it manifests itself severely; in others the same trees show no sign of the problem. Drop has been associated with specific trees: in bad years the same trees show the problem while other, neighboring trees remain problem free. There does not appear to be any spread to existing trees within orchards. It has mainly been reported from Chandler plantings in the Sacramento Valley. In 1995 we began studying set from trees that had off-type and normal Chandler wood grafted to Sunland interstocks. Last year’s data indicated that the off-type scions expressed the off-type disorder. We continued that work this year. We also conducted molecular genetic comparisons of off-type and normal material to address the question of the possible origin of the off type Chandler.

OBJECTIVES

The objectives of the research reported here were to:

1. Continue research on the role of excess pollen load as the cause of PFA, emphasizing Chandler from California's northern San Joaquin and Sacramento valley walnut-growing areas

2. Investigate possible use of Ethrel to remove

3. Examine the off-type Chandler problem
PROCEDURES

Experiments were conducted in San Joaquin, Yuba and Butte counties.

Field procedures to identify PFA and post-PFA flower and fruit drop followed the methods we have been using for the past several years. Briefly, PFA was determined by tagging flowers at bloom and evaluating abscission of the tagged flowers 2 to 3 weeks later, which is prior to the time abscission from lack of pollination would be expected to occur. Approximately two months later the same tagged fruits were recounted to determine post-PFA drop. Although we recognize that the post-PFA drop likely comprises factors other than insufficient pollination, we assume that insufficient pollination is the primary component of this drop and that it can be take as an indicator of problems with insufficient pollen. Twenty-five tags per tree were used in each case. Yield data were taken in the Butte county orchard. For catkin removal experiments, catkins were removed by shaking at the time the catkin buds began to elongate.

Data on off-type Chandler scions was collected on material that had been grafted in 1993 when samples of graft wood were collected from affected and unaffected trees from a Butte county orchard. This material was grafted to Sunland interstock. Each of ten stock tree was dehorned, and affected and unaffected material grafted onto it. Grafts were made so that a single stock tree had two or three limbs each grafted to multiple graft wood taken from one normal and one off-type Chandler. We analyzed set on these scion limbs by counting number of fruits set per terminal (including spur terminals) on the current year’s growth.

Molecular genetic comparisons of off-type and normal Chandler were done using Random Amplified Polymorphic DNA (RAPD) polymorphisms. We collected leaves from off-type Ch anders from three different orchards, extracted DNA and amplified the DNA using the polymerase chain reaction with primers we had previously identified as producing a high level of polymorphic bands among walnut cultivars, and as being diagnostic for Chandler.

Ethrel was sprayed on Serr trees by handgun dilute sprays on November 20, 1995 at two rates plus a check. Rates were: A water check; low, 9 pints/acre (450 gal/acre, 6 gal/tree) or 600ppm; and high, 27 pints/acre (450 gal/acre, 6 gal/tree) or 1800ppm. Catkin drop and nut weight were monitored.

RESULTS

Chandler

In San Joaquin County (Ripon, Orchard F), J. Grant investigated drop in a Chandler orchard (Fisher) with a row of Cisco pollinizers on the downwind side. Catkins were removed by shaking trees in half of the pollinizer row; the other half was left as a check.

Results indicated no significant differences in PFA among treatments or bloom dates. However an interesting trend did emerge in the relationship of PFA to distance from shaken and unshaken
pollinizer trees. For transects in the portion of the orchard where the pollinizers were not shaken there was a positive correlation between PFA and distance from the pollinizers; this correlation was weak for early bloom and slightly negative for the later bloom. For the early bloom period values for the log regressions of PFA vs distance from pollinizers were 0.41 and 0.83 for the shaken and non shaken trees, respectively. Values for later bloom were 0.11 and 0.78. These correlations seem to indicate an effect of pollen reduction in the vicinity of the pollinizers even in this orchard where PFA was generally low.

![Fisher 1996 PFA Early Bloom](image)

![Fisher 1996 PFA Late Bloom](image)

Post PFA drop was uniformly low in the orchard for both treatments and both bloom dates indicating that pollen levels were sufficient to effect pollination throughout the orchard despite the relatively small numbers of pollinizers in the orchard.

<table>
<thead>
<tr>
<th>Post PFA Drop (%)</th>
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</thead>
<tbody>
<tr>
<td>Shake</td>
</tr>
<tr>
<td>Early Bloom</td>
</tr>
<tr>
<td>Late Bloom</td>
</tr>
</tbody>
</table>

In Yuba County, (Rio Oso, Sunrise Orchard), J. Hasey investigated drop in a Chandler orchard (Sunrise). This orchard had a relatively large number of Franquette pollinizers: a pollinizer row every for every eight rows of Chandlers (11.1% pollinizers), and a row of Franquettes on the southern edge of the entire orchard. Thus, no Chandler tree was more than 120 feet from a pollinizer row. We monitored PFA and post-PFA drop without making any effort to reduce pollen load in the orchard.
Transects were marked moving away from the Franquette row at 30, 60, 120, 240 and 480 feet. Three transects adjacent to a row of pollinator trees and three four rows away (maximum distance in this orchard) from the pollinator trees were tagged on two dates. The first tagging was prior to Franquette staminate bloom and the second was during Franquette staminate bloom.

There were no correlations in either drop with distance along the transects. There were also no differences in either drop between trees in rows adjacent to the pollinizers and trees 120 away. Data are summarized in the table where adjacent refers to Chándlers in rows adjacent to the Franquette pollinizers, center refers to trees in rows 120 feet from the Franquette pollinizers, and Adj+Cen refers to both sets of data combined:

<table>
<thead>
<tr>
<th></th>
<th>PFA (%)</th>
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<th>Post-PFA (%)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Adjacent</td>
<td>Center</td>
<td>Adj+Cen</td>
<td>Adjacent</td>
</tr>
<tr>
<td>Early Bloom</td>
<td>34.4</td>
<td>30.6</td>
<td>32.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Late Bloom</td>
<td>40.2</td>
<td>42.2</td>
<td>42.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

In this situation with a large number of pollinizers present, PFA is high especially during the period when pollinizers are shedding. Post-PFA drop is low, however, especially during the period of Franquette pollen shed when it falls to essentially zero.

There were no trends evident with distance either from the southern row of pollinator trees or moving away from the pollinizer rows in the orchard itself. This is likely a consequence of the extremely high number of pollinizers in the orchard and the high pollen loads present throughout. One point that is somewhat difficult to explain is the high PFA during the early bloom period, prior to Franquette pollen shed.

In Butte County, W. Olson investigated PFA and post-PFA drop in two Chandler orchards, one that had Franquette pollinizers (Hennigan Orchard) every tenth row, and one that had no pollinizers (Deseret Orchard). In the Hennigan Orchard, the grower had begun removing Franquette pollinizers by top-working trees to Chandler based on our previous results in this orchard. The Franquette trees that were left in the orchard were shaken to remove catkins. We monitored PFA and post-PFA drop as a function of distance from a row of unshaken Franquette pollinizers.

Flowers were tagged early (1st tagging) and late (2nd tagging) in the Chandler bloom period. The early tagging was prior to Franquette staminate bloom and the late tagging was during Franquette staminate bloom. Yield data were collected at harvest.

Results are shown in the Figures below:
These results show PFA and total drop increasing during the period of Franquette staminate bloom. Post-PFA drop, most likely due to insufficient pollination, is lower than PFA and is somewhat higher prior to Franquette bloom. Total drop throughout the orchard is constant along the transects and higher for the later blooming flowers. This difference is due nearly entirely to the higher PFA experienced in this flower population.

Yield, unlike the drop parameters, showed a strong trend of increasing with distance from the Franquette pollinizers. It is possible that these results reflect PFA events occurring in the upper portions of the canopy which our analyses would not have picked up. We tentatively conclude that trends in PFA as a function of distance from the Franquette pollinizers may be more evident in the upper portions of the canopy than in the lower portions where we actually monitored the phenomenon. Two lines of evidence support this conclusion. First, is the actual yield results shown above. The second is anecdotal evidence from the grower in this orchard who had seen benefits from catkin removal (by shaking) in our previous experiments in this orchard. We had only found minimal effects by monitoring PFA in the lower canopy, but the grower found sufficient differences in his yield to induce him to begin a program of pollinator removal.

The Deseret comparison orchard with no pollinizers showed lower PFA and higher post-PFA drop. The overall data for the two orchards are summarized in the table:
<table>
<thead>
<tr>
<th></th>
<th>Deseret (no pollinizers)</th>
<th>Hennigan (Franquette pollinizers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFA (%)</td>
<td>4.1</td>
<td>25.1</td>
</tr>
<tr>
<td>Post-PFA (%)</td>
<td>33.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Total drop (%)</td>
<td>36.2</td>
<td>39.5</td>
</tr>
</tbody>
</table>

Several points of interest emerge from these data. First, PFA is 70% lower in the orchard without pollinizers during the early bloom stages, and 18.5% lower during the later bloom stage. This is consistent with very low levels of pollen being present in the early stage and increasing levels of pollen in the ambient air as neighboring orchards shed pollen. Second, post-PFA drop, primarily due to lack of pollination, is much higher in the orchard with no pollinizers. The reduction seen in that orchard from the first to the second tagging is consistent with the increase in PFA and, again, is probably correlated with ambient pollen levels from neighboring orchards. Finally, the lack of pollination appears to lead to a greater total drop than that seen in the higher PFA orchard. This final inference must be considered cautiously, however. The Hennigan orchard has greatly reduced pollen levels from the shaking and pollinator removal in the orchard. This has certainly reduced PFA from what one might expect in an orchard where these efforts have not been taken. Also, all of the post-PFA drop in the Deseret orchard may not be attributable to lack of pollination alone; other factors not discussed here may be contributing to the reduced set in these trees.

Chandler off-type (or bull) material was examined on paired grafts of graft wood taken from affected and unaffected trees. Results are shown below.
We determined the number of nuts per terminal shoot for 10 or more off-type and normal shoot on each stock tree on June 12, 1996. In all but one case (tree 8) the normal scions had greater number of nuts when compared to the off-type scions on that tree. Overall normal scions had 2.19 times as many nuts per terminal shoot as did the off-type scions.

These results are comparable to results of similar experiments conducted last year on these same trees when we found higher set on the normal scions. These findings confirm our earlier conclusion that the off-type disorder is graft-stable and that using existing off-type Chandler material for graft wood must be avoided.
In addition we conducted genetic analyses comparing normal and off-type Chandler in order to gather information on the probably nature of the off-type Chandler material. Earlier research using isozyme analyses indicated that the off-type was not different from normal Chandler. Using the higher level of resolution attainable by RAPD analysis we obtained similar results. No differences were evident between Chandler and any of the off-type samples. These results are consistent with the hypothesis that the off-type originated as a bud-sport mutant of Chandler and that it has been propagated by grafting. While the results do not absolutely rule out the possibility that the off-type is a different cultivar or selection that had been confused with Chandler early in the distribution, this possibility is increasingly unlikely in the face of these results. The figure above illustrates representative gels showing 10 different markers, each highly polymorphic among walnut cultivars. Typical of RAPD analysis, some lanes are inconclusive in these gels, however, the analyses were repeated five times which was sufficient to reproduce results from each of the 10 markers. Chandler can be readily distinguished from other walnut cultivars using these markers.

**Ethrel Removal of Serr Catkins**

Bill Coates investigated the effect of Ethrel on Serr catkin removal. For both high and low Ethrel treatments, leafing was delayed relative to the check. Catkins drop occurred in January and February. Number of catkins were determined before bloom in March. The low rate delayed leafing by about 4 days; the high rate delayed leafing for 10 days. Results are summarized in the table:

<table>
<thead>
<tr>
<th>Ethrel (ppm)</th>
<th>Leafing Delay (days)</th>
<th>Catkins/Limb</th>
<th>Nut Weight (g/nut)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>0</td>
<td>0</td>
<td>104.0</td>
</tr>
<tr>
<td>Low</td>
<td>600</td>
<td>4</td>
<td>57.3</td>
</tr>
<tr>
<td>High</td>
<td>1800</td>
<td>10</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Previous results indicated that nut size was reduced following Ethrel removal of catkins, however, those experiments were carried out on individual limbs and it is likely that the nuts on the treated limbs were at a competitive disadvantage relative to other nuts on the tree due to the delay in leafing and bloom times that resulted from the treatment. The present results from whole tree sprays indicate that Ethrel sprays may be a useful method for removing catkins without sacrificing nut size.

There are potential benefits from removing catkins with Ethrel as opposed to shaking trees. In some years (late rains, especially in orchards with heavy clay soils) it may not be possible to get
shaking equipment into the orchard to do the shaking. Removal of catkins by fall Ethrel spray can insure against this. In addition, the delay in leafing may also be an advantage in areas where walnut blight is a problem for early cultivars in years when there is late-spring rain. Delaying leafing by up to 10 days may shift the season sufficiently later to minimize blight.

Based on these positive results and the potential advantages that may accrue from removing catkins by fall sprays of Ethrel, we will be expanding these experiments next year. During the fall of 1996 we conducted large-scale experiments in Tulare and Butte counties as well as repeating the single-tree experiments in San Benito county.