Pistillate Flower Abscission in Turkish Walnut Cultivars and Its Reduction by AVG

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Abstract: Level of pistillate flower abscission (PFA) in Turkish walnut cultivars was investigated in this study. Emasculated and bagged female flowers were pollinated with 5%, 50% and 100% pollen concentrations at receptivity. Control flowers left for open pollination. In addition, a whole tree was sprayed with 125 ppm ethylene inhibitor AVG (amino ethoxy vinyl glycine) when anthesis reached to 5-30% and a control tree was not treated for each cultivar. Number of aborted flowers at diameter of 3-4cm was counted and percent PFA was calculated. The results showed that Turkish walnut cultivars had medium (65.4%) to high (100%) levels of PFA including the leading cultivars 'Şebin' and 'Bilecik' (94.8% and 93.4% PFA, respectively). Application of AVG reduced PFA from 82.4% to 43.6% in average and increased fruit set significantly in all cultivars except 'Şen-1'.

Keywords: Walnut, PFA, fruit set, amino ethoxy vinyl glycine, AVG

Introduction

Walnut (*Juglans regia* L.) is a monoecious and wind pollinated species. Pistillate flowers are produced terminally on shoots while staminate flowers (catkins) are borne laterally on one-year-old branches. Many fruit species exhibit flower and fruit drops which usually associated with competition phenomenon or lack of pollination. Walnuts present female flower drops called Pistillate Flower Abscission (PFA) (Catlin et al. 1987). Female flowers become receptive very shortly after emergence from the shoot apex when two stigma lobes begin to separate. However, ovary stops growing at 3-4mm in diameter, become necrotic, get brown and mumificated. These flowers drop with the peduncle attached from the shoot 2-3 weeks later (Catlin &

Polito, 1989, Polito et al, 2005, Rovira & Aleta, 2006). In early studies, no association could be made with any known physiological, cultural, pathological or entomological influence (Catlin et al. 1987). Later, reports of Por & Por (1990) and McGranahan et al. (1994) showed that excess pollen was involved in PFA. Recently, the physiological mechanism leading to PFA was uncovered. When a pistillate flower receives high pollen load, pollen tubes growing down the style induce high rate of ethylene biosynthesis activating pre-formed abscission zones and resulting in flower abscission (Beede & Polito, 2003, Polito et al. 2005, Johnson et al. 2006, Lemus et al. 2007).

Mechanically shaking catkins from pollenizer cultivars and main cultivar or removal of pollenizer rows altogether have been suggested to minimize PFA in existing orchards (Sibbett et al. 1995). An ethylene inhibitor amino ethoxy vinyl glycine (AVG) has been tested on walnuts to reduce PFA. The results showed increased yields especially on 'Serr' cultivar (Beede & Polito, 2003, Lemus et al. 2007). Severity of PFA varied among cultivars and 'Serr' was more seriously affected than others were. The yield could be reduced as much as 90% (Catlin & Olson, 1990). Many observations made in some countries such as Chile, France, Hungary, Spain, USA and Iran demonstrated that PFA exist in nearly all commercial cultivars (Rovira & Aleta, 1997, 2006, Hassani et al. 2006, Lemus et al. 2007). However, tendency of Turkish cultivars to PFA is not known.

The objective of this study was to determine the level of PFA in Turkish walnut cultivars and to prevent PFA by applying ethylene biosynthesis inhibitor AVG.

Materials and Methods

Sixteen years old walnut trees of 11 Turkish cultivars and 'Serr' cultivar located at Atatürk Central Horticultural Research Institute, Yalova, Turkey were used in the study [Table 1].

Determination of the level of PFA in cultivars:

Newly coming shoots with emerging female flowers were bagged with Tyvek bags before receptivity after emasculation. Female flowers were pollinated with 5%, 50% and 100% of walnut pollen when stigmas separated and reached to receptivity. Pollen mixtures were prepared with talc powder before application. Pollen numbers in each of concentration was determined under microscope. Control flowers left for open pollination. Dropping flowers due to PFA were distinguished by visual appearance (Rovira & Aleta, 2006); female flowers stopped growing at 3-4mm in diameter, became necrotic, got brown, and mumificated. The most distinct feature was the dropping of flowers with the peduncle attached to it from the shoot. Drops due to competition within flowers of the same inflorescence were different that flowers' detachment occurred at the basis of ovary and the flowers dropped without peduncle. Number of healthy flowers and abscised flowers showing PFA symptoms were counted and the percent PFA was calculated after 3 weeks of pollinations. For each treatment, 25 bags were used on each of three trees. Number of female flowers in bags varied since multiple flowers could be produced at top of shoot apex.

Determination of effect of ethylene inhibitor on reduction of PFA:

AVG (amino ethoxy vinyl glycine) was used as an ethylene inhibitor. A whole tree was applied with 125 ppm of commercial product Retain® (Valent BioSciences, USA) when female flowers reached to 5-30% receptivity (Venburg et al. 2008). Second tree was not treated and used as control. Three branches were marked and approximately 100 flowers were counted on trees. Number of healthy and abscised female flowers due to PFA was counted and percent PFA was calculated after 3 weeks of AVG application. Cultivar 'Kaplan-86' gave inconsistent results that it was excluded from this experiment.

In both of the experiments, the second set of counts was made nine weeks after pollination/AVG application to assess fruit set which gave the yield. Drops occurred at that period due to both PFA and other causes of drop such as nutritional deficiencies and competition phenomenon.

Results and Discussion

Determination of the level of PFA in cultivars:

Percent PFA and percent fruit set values of cultivars, which were determined 3 weeks, and 9 weeks after pollination with different pollen concentrations, respectively are shown in Table 1. When female flowers pollinated with the lowest concentration of pollen (5%) PFA was the lowest (48.6%). As pollen concentration increased to 50% and 100% PFA incidence increased to 54.1% and 72.7%, respectively. Pollinations with 100% pollen concentration gave similar to open pollination. We counted the pollen number in the same amount of pollen sample, which was applied to the flowers under microscope. The average number of pollen grains was 17.6, 47.7 and 343.8 in pollen samples of 5%, 50 and 100% of concentrations, respectively. These findings indicated a positive relationship between pollen load and level of abortion of pistillate flowers as suggested by Por & Por, (1990) and McGranahan et al. (1994). Kaveckaja & Tokar (1963) reported that 10-18 pollen grains were necessary for regular fruit set on stigmas. McGranahan et al. (1994) counted 179 pollen grains on normally developing stigmas while there was more pollen grain (322) on flowers, which affected by PFA (ovary diameter was 3-4mm) in cultivar 'Serr'. The researchers concluded that there was significant negative correlation between pistil size and number of pollen grains. Dose response curves of Polito et al. (1996) indicated that pollen doses in the range of 70-100 grains per pistillate flower would induce 50% abortion in pistillate flowers; higher levels of pollen resulted in greater losses.

The counts made after 9 weeks from pollinations showed that average fruit set was similar in all treatments being 17.6 to 20.2% that was unexpected. Although the reason for this is not known we thought that drops occurred at that period was due to both PFA and other causes of drop such as nutritional deficiencies and competition phenomenon. Polito et al. (2005) considered the drops at late stage primarily due to a lack of effective pollination. Rovira & Aleta (2006) reported that unfertilized flowers could reach advanced development stage without fecundation; they reach an appearance of small fruits, up to 8-10mm of diameter. They may remain attached for three weeks or longer. On the other hand, recently fertilized nut drops are usually attributed to nutrients competition and at this stage, fruit size is about 12-18mm in diameter.

Cultivar	%5 pollen		%50 pollen		%100 pollen		Open pollination	
	PFA (%)	Fruit set (%)	PFA %	Fruit set (%)	PFA (%)	Fruit set (%)	PFA (%)	Fruit set (%)
Y-1	21.9	25.1	28.5	35.7	65.7	22.6	87.9	10.6
Y-3	24.1	27.8	49.2	10.3	72.2	10.7	67.9	25
Y-4	54.9	0.0	76.6	0.0	83.6	12.8	68.8	9.3
Şebin	59.4	17.4	54.1	26.9	62.8	30.6	88.4	11.6
Bilecik	51.9	34.6	55.1	34.9	71.8	19.5	88.9	7.6
Tokat-1	63.1	29.5	68.6	13.8	54.5	27.3	63.0	37.0
KR-1	55.5	16.7	33.3	37.5	77.1	19.4	81.7	10.1
KR-2	16.8	48.1	16.0	36.0	85.0	10.5	85.2	13.7
Şen-1	47.6	17.0	54.6	36.5	77.8	18.6	53.8	30.8
Şen-2	77.1	0.0	73.6	2.1	82.8	0.0	59.8	18.9
Kaplan-86	83.3	12.5	93.3	0.0	78.8	21.2	100.0	0.0
Serr	26.3	14.1	46.8	13.7	60.9	33.6	53.5	36.5
Average	48.6	20.2	54.1	20.6	72.7	18.9	74.9	17.6

Table 1. PFA (%) and fruit set (%) values of Turkish walnut cultivars and 'Serr' which were determined 3 weeks and 9 weeks after pollination with different pollen concentrations, respectively.

Level of PFA in the cultivars to varied that it was the highest in 'Kaplan-86' followed by 'Bilecik', 'Şebin', 'Y-1' and 'KR-1' when the female flowers left for open pollination [Table 1]. The least affected cultivars were 'Serr' and 'Şen-1'. These two cultivars also had about average values for PFA after pollinations with different pollen concentrations. In addition, these cultivars had very high fruit set values after open pollination. 'Şen-1' might have lower levels of PFA, while the results were unexpected for 'Serr' since it is highly affected by PFA. We observed that 'Serr' blooms later than most of Turkish genotypes. Thus, airborne pollen was drastically lower at the experimental site, which could result in lower PFA and higher fruit set compared to other cultivars. Researchers found that response of the cultivars to PFA varies with cultivars. 'Chico' showed the lowest levels of PFA, and 'Chandler' and 'Vina' were intermediate (Polito et al.1996). Similarly, Spanish selections were the most affected by PFA showing 73.4% of flower loss while Chilean selections had only 6.8% flower drop due to PFA. Californian and French cultivars showed medium values but closer to Chilean selections (Rovira & Aleta, 2006). PFA was found between 11 to 92% among Iranian genotypes (Hassani et al. 2006).

Determination of effect of ethylene inhibitor on reduction of PFA:

Percent PFA and percent fruit set values of the cultivars that were determined 3 weeks and 9 weeks after application of AVG, respectively are shown in Table 2. In general, PFA was very high (82.4%) in open pollination conditions (control). AVG is an ethylene biosynthesis inhibitor and it has been applied predominantly to control abscission and ripening in apples. Use of AVG in walnuts to reduce PFA is a novel application. 125 ppm of AVG has been suggested as an adequate concentration to improve fruit set. In our study AVG treatment was effective on reducing PFA and increasing fruit set compared to the control. PFA was reduced from 82.4% to 43.6% and the reduction was about 50 percent. Fruit set was increased from 16.3% to 46.8% and the increase was almost three fold. Some cultivars such as 'KR-1', 'KR-2' and 'Tokat-1', benefitted more from AVG application than others that they had the lowest PFA (12 to 18%) and the highest fruit set (84 to 87%) values. Although AVG application reduced PFA in 'Serr', fruit set was higher than the control. 'Serr' blooms later than most of Turkish genotypes, which are fruitful on terminal shoots. Our observations showed that terminal buds responded quickly to warm temperatures and development of pistillate flowers progressed fast in Turkish genotypes. When female flowers of 'Serr' reached to receptivity airborne pollen was drastically lower at the experimental site, which could result in higher fruit set in open pollination conditions. Polito et al. (2005) reported 57 to 70% yield increase in California and Lemus et al. (2007) obtained 35 to 83% yield increase in Chile in 'Serr'.

There was no improvement neither for reduction in PFA nor for increase in fruit set in 'Şen-1'. The results indicated that this cultivar had lower levels of PFA than the others did. Buchner et al. (2006) reported similar results that AVG application did not improve yield in 'Chandler' cultivar. Cultivars 'Y1', 'Y3' and 'Y4' (Yalova series) have been widely planted in early plantings in 1980s and 1990s in Turkey. However, there have been

Cultivar	AVG Application		Control	
	PFA (%)	Fruit set (%)	PFA (%)	Fruit set (%)
Y-1	45.7	54.3	87.0	12.0
Y-3	31.5	52.3	68.6	25.4
Y-4	47.6	43.8	98.1	0.0
Şebin	71.1	27.9	94.8	5.2
Bilecik	74.5	16.7	93.4	3.2
Tokat-1	12.0	87.0	66.7	33.3
KR-1	17.0	71.7	82.9	11.4
KR-2	18.0	64.0	100.0	0.0
Şen-1	65.0	27.8	65.4	28.9
Şen-2	46.0	28.7	80.4	10.7
Serr	51.4	40.5	68.7	49.0

Average	43.6	46.8	82.4	16.3

Table2. PFA (%) and fruit set (%) values of Turkish walnut cultivars and 'Serr' which were determined 3 weeks and 9 weeks after 125pp AVG application, respectively.

complaints about low yields in these cultivars. Our results showed that they had high levels of PFA and AVG significantly reduced PFA and increased fruit set. One of the important findings of this study was to show PFA levels of 'Şebin' and 'Bilecik' cultivars, which have a special place in Turkish walnut cultivation. Recently, there have been hundreds of orchards established with these two cultivars in the country; the former is main cultivar and the latter is pollenizer. The results highlighted that both cultivars are highly affected by excess pollen load and PFA occurs in large extend. On the other hand, AVG application was very effective in reducing PFA that fruit set increased 3 to 5 folds.

Conclusions

PFA is associated with excess pollen load on stigmas in walnuts. Excessive pollen tubes growing down the style of the female flower produce excessive amounts of ethylene, which is associated with organ senescence. Elevated ethylene levels are cause of flower abortion (Beede & Polito, 2003). PFA can be minimized in existing orchards by either mechanically shaking catkins from pollenizers, from both pollenizers and main cultivar, or removal of pollenizer rows altogether (Sibbett et al. 1995). However, in many cases these applications are not practical. Although the results are preliminary, we could be able to show that Turkish walnut cultivars had medium to high levels of PFA. Application of 125ppm AVG could effectively reduced PFA in all cultivars including the leading ones 'Şebin' and 'Bilecik' except 'Şen-1'.

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