Dust Interferes with Pollen–Stigma Interaction and Fruit Set in Pistachio *Pistacia vera* cv. Kerman

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Abstract. Springtime flail mowing of row middles for weed control in California pistachio (*Pistacia vera* L.) orchards blows dust into the leafless canopy if it occurs during bloom. The effect of dust on pistachio pollination and fruit set is unknown. Rachises were bagged prebloom and hand pollinated with pollen and dust mixtures at 1:0, 1:1, 1:4, 1:16, and 0:1 volume/volume ratios on five successive days. The 2016 and 2017 trials demonstrated that the inflorescences treated with a high pollen:dust ratio (0:1, 1:4, and 1:16) had significantly lower split nut rates (commercially less profitable) compared with low dust ratio tests (1:0 and 1:1). Our results also showed that dust damaged both pollen viability and stigma quality, particularly if contaminated with herbicide residues (GlyStar® Plus and Treevix®). Decreased yield was a function of decreased fruit set; increased embryo abortion, parthenocarpy, or both; and a lower split nut percentage. The GA₃ content in flowers of both the pollen and dust treatments was significantly higher than that in nonpollinated flowers, suggesting dust stimulated parthenocarpy, resulting in empty nutshells, “blanks” at harvest.

Pistachio (*Pistacia vera* L.) is a deciduous dioecious tree grown commercially in the high desert and Mediterranean climates of Iran, California’s Central Valley, Turkey, Sicily, and Australia. Pistachio is an anemophilous species; air currents carry pollen from the male tree to the female tree. Both the male staminate and female pistillate inflorescences are panicles composed of hundreds of

individual small flowers, the rachis. The florets of both female and male flowers are apetalous with fully exposed stigmas and anthers vulnerable to desiccating winds, extreme temperatures, and rain (Ferguson et al., 2005).

The receptivity of the stigma of an individual pistachio female floret is 2–3 d; thereafter, the stigma surface senesces and cannot be pollinated (González et al., 1995; Hedhly et al., 2003). The amount of viable pollen loaded on the stigma is the first step in pollination (Wilcock and Neiland, 2002). Acar and Eti (2008) reported 14 or 15 pollen grains per stigma is adequate for pollination in pistachio. On germination, the pollen tube grows the length of the style to the micropyle, the opening in the ovule’s outer layer. There, it enters the embryo sac (female gametophyte) and completes fertilization. Successful fruit set is collectively a function of stigma condition, pollen germination and tube growth, ovule longevity and the effective period between pollination (pollen landing on the stigma), growth of the germinated pollen tube through the style, and fertilization of the ovule (Herrero and Arbeloa, 1989; Kalinganier et al., 2000; Ortega et al., 2004; Zhang et al., 2018).

The combination of dry conditions, wind, and flail mowing during pollination has been observed to generate considerable dust. The diameter of orchard dust particles is between 1 and 100 µm, whereas the surface length of the female stigma and the diameter of pollen grains are ≈500 and 20 µm, respectively. Field dust has been demonstrated to injure the leaf stomata and spongy parenchyma beneath, preventing photosynthesis and inhibiting pollen loading on the floral stigmas, resulting in poor pollination (Sett, 2017; Waser et al., 2017). The female flower’s acidic stigmatic secretions that facilitate pollen hydration and germination can also be damaged by alkaline dust (Sett, 2017). McArtney et al. (2006) reported sulfur liming at bloom inhibited pollen tube growth and subsequently reduced fruit set in apple (*Malus domestica*).

There is little information on how dust affects the pollen and pistils, or their interaction. Most reports focus on the pollen–stigma interactions of the self-incompatible or foreign pollen (Goring, 2018; Hiscock et al., 2002). The pollen coat, composed of lipids and proteins, contains some signaling molecules such as pollen coat B-class (PCP-Bs) and S-locus proteins that are required for stigmatic receptors to trigger pollen recognition and start papillar responses (Doughty et al., 2000). The exotic PCP-Bs carried by the incompatible pollen have been reported to prevent pollen hydration in the Brassicaceae (Wang et al., 2017). On the attachment of the pollen and stigmas, vesicle-like structures in the papillae cells were observed moving toward and fusing with the papillar plasma membrane at the pollen–stigma interface. These vesicles transport the aquaporins and lipases (e.g., pectinase) that facilitate water distribution that precipitates grain hydration, and cell wall expansion that facilitates the pollen tube penetrating the papillar layers (Elleman et al., 1992). However, these stigma reactions have not been observed in the flowers pollinated by self-incompatible pollen grains (Safavian and Goring, 2013). We hypothesized that dust, as an abiotic substance, will not provoke signaling-related biochemical reactions and produce the same effect as an incompatible pollen.

We addressed the following questions. First, does dust on the stigmatic surface reduce pollination and fruit set in pistachio? Second, do herbicide residues in the dust harm pollination and yield as a direct effect of the herbicide harming the stigma and pollen? Third, does dust influence pistachio nut growth and shell splitting? The final value of harvested pistachios is a function of the size and percentage of successfully harvested filled and split nuts. Blank, partially filled, and unsplit nuts can be successfully harvested or remain on the tree; in either case, yield and quality, and, therefore, net return suffer. The objective of this study was to investigate if orchard dust harms pistachio pollination and how.

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Materials and Methods

Plant materials. In late Mar. 2016 and 2017, the pollen from male ‘Peters’ pistachio trees was collected from early blooming orchards. The pollen was dried at room temperature for 3 d and stored at 24.8 °F. The stored pollen was used within 2 weeks of collection. Dust was collected from the soil surface in the experimental field and preprocessed at 300 °F for 1 h to inactivate any pollen in the dust. Before bloom, clusters with flowers at prebloom bud extension were isolated and covered with white nonwoven polypropylene bags.

Pollen viability test. The pollen viability of the pollen and 1:0, 0:1, 1:1, 1:4, 1:16, and 1:1 volume/volume (v/v) ratios of pollen and dust and herbicide-contaminated dust were tested by

AmpaZ30 (Amphasys AG, Root, Switzerland) (Heidmann et al., 2016). The 1:1 herbicide-contaminated treatment was the pollen and dust mixture in which the 300 °F-processed dust was presoaked with commonly used herbicides GlyStar® Plus (Albaugh LLC, Ankeny, IA) and Treevix® (BASF, Durham, NC) with label-recommended concentrations and dried at 75 °F under a fume hood. The Amphasys, an impedance flow cytometer, uses a laboratory-on-chip technology to measure pollen viability by imparting an electrical charge on single cells passing through the microfluidic channel on a semidisposable chip. The viable pollen percentage of each sample was tested on 4500 particles of pollen, dust, or pollen and dust mixtures per group. Each group had a heat-treated (100% dead particles) sample set as the control.

Fruit set and yield investigation. In 2016, bagged Kerman inflorescences were hand pollinated with a brush on three successive days with dust, pollen, and a pollen and dust mixture (v:v = 1:1) during bloom. A bagged control was simultaneously maintained. The nuts per cluster were counted and weighed at harvest, and the percentage of blank nuts were also recorded.

In 2017, the pollen and dust 1:0, 0:1, 1:1, 1:4, and 1:16 mixtures and the 1:1 herbicide-contaminated, plus the 1:1 treatment (dust was applied first and pollen was brushed on the same flower 4 h later) were applied to fresh isolated flowers. Bagged clusters were hand pollinated with these seven pollen and dust mixtures at the different ratios on five successive days. Fruit set, blanks, nut drop, and nut split percentage of the cluster units were recorded. Fruit set (%) was evaluated based on per cluster flower and nut numbers counted 10 weeks postbloom. Nut drop (%) was calculated as the number of nuts dropped between 10 weeks postbloom and harvest. Nuts without fully developed embryos at harvest were graded as blanks and nuts with naturally opened shells graded as split nuts. Nonsplit nuts have less commercial value as shelled out ingredient product. Data were analyzed using the general linear model program of the SAS statistical analysis followed by Duncan’s multiple range test at $P = 0.05$.

Stigma structure observation. The floral stigma structures treated with the pollen, dust, the 1:1 herbicide-contaminated treatment, and the bagged control were observed with a scanning electron microscope (SEM). The samples were fixed in Karnovsky’s solution (2.5% paraformaldehyde + 2.0% glutaraldehyde in 0.08 M sodium phosphate buffer, pH 7.2) for a minimum of 24 h and washed in 0.1 M sodium phosphate buffer, pH 7.2. The samples were then dehydrated in a graded ethanol series at 30%, 50%, 70%, and three washes of 95% and 100% (all 15 min each). The samples were then dried in a Tousimis SuperCritical Auto Samdri 931.GL Critical Point Dryer (Tousimis, Rockville, MD). The samples were mounted onto aluminum stubs and coated with gold in a PELCO SC7 sputter coater (Ted Pella, Inc., Redding, CA). The samples were viewed on a Philips XL30 SEM (FEI Company, Hillsboro, OR, made in Eindhoven, Netherlands).

Gibberellic acid (GA_3) estimation. Florets of the pollen- and dust-treated flowers and the bagged control were sampled and tested for GA_3 concentrations using an HPLC-MS. For each sample, a total of 0.10 g of pulverized tissue was weighed and extracted overnight at 4 °C in 0.5 mL of methanol. The supernatant was then transferred to new tubes, and the pellet was extracted again overnight at 4 °C in 0.5 mL of methanol. This second methanol extract was then combined with the first supernatant to yield 1 mL of methanol extract for each sample. The samples were

Fig. 1. Fruit set of clusters with pollen or dust application at bloom. Clusters with flowers at the green tip stage were isolated and covered with white nonwoven polypropylene bags. (A) Fruit set in April of flowers with pollen applied at bloom. (B) Fruit set in April of flowers with dust applied at bloom. (C) Fruit set in July of flowers with pollen applied at bloom. (D) Fruit set in July of flowers with dust applied at bloom.

Table 1. Viable pollen rate, fruit set, nut drop, blank, and split rates of trials with pollen (P) and dust (D) mixtures in different ratios and treatments with male cultivar Peters and female cultivar Kerman in 2017.

Treatments	Viable pollen rate (%)	Fruit set (% determined 10 wk postbloom)	Nut drop (% determined at harvest)	Blank rate (%)	Split rate (%)
Control		3.6 C	46.4 B	83.3 A	0.0 B
Pollen	24.4	18.2 AB	11.5 BC	10.9 B	51.7 A
Dust	0.00	3.3 C	0.0 C	80.0 A	10.0 B
P:D 1:1	19.6	17.0 AB	10.4 BC	16.5 B	61.7 A
P:D 1:4	11.0	24.2 A	15.4 BC	42.6 AB	30.9 B
P:D 1:16	4.2	11.5 BC	17.0 BC	39.3 AB	31.6 B
1:1 toxic	2.5	5.2 C	100.0 A		
P-D 1-1		11.9 BC	36.8 BC	25.6 B	43.6 AB

Values within the same column followed by the same letter are not significantly different according to Duncan’s test ($P = 0.05$).

Control: the treatment in which inflorescences were isolated in white nonwoven polypropylene bags to avoid natural pollination and later were left without hand pollination.

The 1:1 toxic (herbicide-contaminated) treatment: dust of the 1:1 pollen:dust mixture was presoaked with herbicides GlyStar® Plus and Treevix®.

The P-D 1:1 treatment: dust was applied first and the pollen was brushed on the same flower 4 h later.

Fig. 2. Coefficients of determination for 'Peters' pistachio pollen viability to 'Kerman' pistachio fruit set (A), blank rate (B), and split rate (C) in 2017.

analyzed on a Shimadzu (Columbia, MD) LC-20AD-based high-performance liquid chromatograph with a photodiode array detector and an LCMS-2020 mass spectrometer running a 0.2% acetic acid acidified water-methanol gradient separation, following the methods of Wallis and Chen (2012) with a Phenomenex (Torrance, CA) Onyx monolithic C18 column. A GA_3 standard was run to derive a standard curve for quantification by peak area at 280 nm and to identify the retention time of GA_3 . A selective ion mode was used to target the GA_3 peak at this point by quantifying its ion,

identified by molecular mass, as coelution with other compounds occurred.

Results

Dust and nut yield. Our investigations in 2016 and 2017 demonstrated that applying dust at bloom to female flowers sharply decreased fruit set compared with flowers dusted with pollen. At 2016 harvest, the nuts on rachises treated with dust were virtually all blank, fully expanded shells without developed kernels. The rachises receiving dust produced 32% fewer nuts by count and 38%

less in weight relative to the rachises with applied pollen. To specify how dust influences yield, the pollen and dust mixtures with volume ratios at 1:0, 0:1, 1:1, 1:4, and 1:16 as well as the 1:1 herbicide-contaminated mixture and 1:1 treatments were applied to fresh flowers in 2017 (Fig. 1). Generally, the higher the dust levels of the mixtures, the lower the percentage of fruit set and nut yield. In pistachio, the nut split rate is a positive index of yield and nonsplit nuts are commercially less profitable. The nut split rate of the 1:1 pollen:dust treatment was significantly higher than the 1:4 and 1:16 treatment. While comparing the 1:4 and 1:16 applications, the fruit set of the 1:4 mixture was statistically higher than that of the 1:16 mixture. The percentage fruit set produced by dust alone was statistically significantly lower than the percentage produced by the pollen and the 1:1, 1:4, and 1:16 pollen:dust mixtures (Table 1). Compared with the mixed dust and pollen applications (e.g., 1:4), the fruit set of the 1:1 (delay in pollination after dust exposure) treatment was significantly lower. This suggested that dust blocked the interface for the pollen-stigma interaction, and fewer pollen grains were able to generate pollen tubes to complete fertilization. Among the treatments, crop production of the 1:1 herbicide-contaminated trial was the worst, with less than 5.2% fruit set after bloom and 100% nut drop before the harvest.

Dust and pollen viability. In Table 1, the "Viable pollen rate" column demonstrates that as the dust:pollen ratio increases, pollen viability decreases. The pure pollen, and the 1:1, 1:4, and 1:16 pollen and dust treatments had viable pollen percentages of 24.4%, 19.6%, 11.0%, 4.2%, and 0.0%, respectively. The blanking percentage of the dust treatment was as high as 80%, whereas that of the pure pollen was 11.0%. The coefficient of determination, R^2 , of pollen viabilities to the blank and split percentage were 0.83 and 0.81, respectively (Fig. 2). This strong relationship suggested that poor pollen quality could be an essential factor causing high blank and low split percentages in pistachio production. The percentage of viable pollen grains after application of the 1:1 herbicide-contaminated mixture was 2.5%, and that of the fruit set was 5.2%, both significantly lower than that of the pure pollen and 1:1 treatment. This demonstrated that herbicide introduced into the pollination process with dust can destroy pollen viability.

Dust and stigma quality. The stigma surface length of a pistachio floret is $\approx 500 \mu m$ (Fig. 3). The spherical pollen (Fig. 3A) and dust (Fig. 3C) were both $20 \mu m$ in diameter. After the dust application (Fig. 3B), the stigma wilted. With the herbicide-contaminated 1:1 treatment (Fig. 3D), the stigma's papilla cells disappeared. In addition to the pollen damage, our results demonstrated that dust decreased the effective pollination period and that herbicide residues in the dust degraded the stigma. The combination of poor pollen viability and a deformed stigmatic structure

Fig. 3. Stigma structures of pistachio flowers with (A) pollen applied (scale bar = 200 µm) pistachio pollen was spherical with a diameter at around 20 µm; (B) dust applied (scale bar = 500 µm), the stigma was wilted; (C) dust applied (scale bar = 10 µm), the stigma surface was fully covered with dust flakes; (D) 1:1 pollen:dust mixed with herbicides applied (scale bar = 200 µm), the stigma's papilla cells disappeared; (E) 1:1 pollen:dust mixed with herbicides applied (scale bar = 50 µm); and (F) control without dust applied (scale bar = 200 µm), observed under a scanning electron microscope.

paralleled the decreased fruit set and increased nut drop of the 1:1 herbicide-contaminated dust application.

Dust and flower GA_3 contents. Our results (data not listed) demonstrated the GA_3 content in the flowers of the pollen and dust treatments was 20.37 ($\pm$ SE 0.644) and 20.93 ($\pm$ SE 0.089) µg/g, respectively, 1.52 and 2.08 µg/g higher than that in nonpollinated flowers.

Discussion

A pistachio pistil has a three-lobed stigma originating from one primary carpel and two secondary carpels (Hormaza and Polito, 1996; Shuraki and Sedgley, 1997). Hormaza and Polito (1996) observed that the two secondary carpels sometimes do not develop stigmas in 'Kerman' pistachio. The 'Kerman' flowers we investigated in this research produced stigmas with two lobes: one large lobe and one small spatulate lobe. The dust loaded on the stigma was of the same size as the pollen, covered the stigma surface, and

blocked the interface between the pollen and the papillae cells (Fig. 3C).

In pistachios, pollen-induced parthenocarp, fruit set without fertilization, produces a fully expanded pericarp (shell) without an embryo (kernel), which is known as a "blank" nut in the industry (Ferguson et al., 2005). In Table 1, dust treatment had a comparably higher blank rate than pure pollen and 1:1 pollen:dust mixture treatments. This strongly suggests that the dust precipitated parthenocarp. Moreover, the proportion of the viable pollen in the pollen:dust applications and the applications containing herbicide were lower than that of the pure pollen and higher percentage pollen applications (Table 1). These results, combined with the correlation analysis (Fig. 2), demonstrated that pollen viability strongly influences fruit set and embryo growth. This confirms that although the viable pollen is essential for fertilization, dust or the nonviable pollen can induce parthenocarp. Our result is consistent with earlier grape, *Vitis vinifera* (Alva et al., 2015), apple, and european pear *Pyrus com-*

munis L. (Visser and Clara Marcucci, 1984) research demonstrating that parthenocarp can be stimulated by defective or self-incompatible pollen.

The self-incompatible pollen can activate the expression of genes related to gibberellin stimulus that promotes fruit set without pollination in mandarin (*Citrus reticulata*) and grape (Alva et al., 2015; Miao et al., 2013). Exogenous gibberellic acid (GA) applications generate parthenocarp in European pear production. European pear is a naturally parthenocarpic species, and GA applications increase yield when spring frosts damage flower styles (Yarushnykov and Blanke, 2005). Our current research demonstrated that the GA_3 content in flowers of both the pollen and dust treatments was higher than that in nonpollinated flowers. Compared with the nonpollinated treatment, this increase in GA_3 levels in the dust treatment suggested dust can stimulate parthenocarp, producing the unexpectedly high blank percentages and the decreased yields we observed.

Among the treatments, the viable pollen rate and fruit set were lowest in 1:1 herbicide-contaminated trial, which had a 100% pre-harvest nut drop (Table 1). The stigmatic structure of the styles was damaged in that the herbicide mixture caused partial papillae decomposition (Fig. 3D–E). Papillae decomposition was not observed with the pure dust application. Yi et al. (2003) reported that in almonds (*Prunus dulcis* Mill.), stigmatic papillae wrinkled, distorted, and collapsed following fungicide contamination. Similarly, in soybean (*Glycine max*), the herbicide lactofen activated singlet oxygen generation, inducing cell death (Graham, 2005). Another hypothesis is that the self-incompatible pollen interacts with pistil S-determinants to trigger the inhibition of pollen growth via a Ca^{2+} -dependent signaling network, resulting in the programmed cell death (PCD) observed with foreign pollen in corn poppy (*Papaver rhoeas*). Self-incompatible pollen has also been reported to accelerate PCD of papillae cells in olive (*Olea europaea* L.) (Irene et al., 2010; Wheeler et al., 2010). However, there are few reports in the literature on the effects of abiotic particles on stigma integrity or papillae structure. Based on the data presented here, we hypothesize that dust with herbicide residues at bloom caused stigma papillae cell death, which could be a phenomenon of signaling regulation of PCD.

There are multiple reports that particulate pollution (e.g., haze) reduces plant growth by larger dust smothering the stomata and smaller particles entering the substomatal cavity contacting and damaging the spongy parenchyma (Farmer, 1993; Rai et al., 2010; Sett, 2017). However, it is not known if dust particles invade the internal structure of the flower style.

Pollination is a delicate progress involving a series of pollen–pistil interactions or rejections, including pollen–papillae interface formation (called 'foot'), calcium gradients,

actin networks, and polarized secretion (Dresselhaus and Franklin-Tong, 2013; Goring, 2018). For example, the S-receptor kinase in papillae cells activated by S-locus protein 11/S cysteine-rich (SP11/SCR) of the compatible pollen could trigger the downstream self-incompatibility pathway in the stigmatic papillae to prevent the development of foreign grains (Doucet et al., 2016). With such a mechanism, it appears dust is not able to penetrate the floral style. However, there could be an exception in that the stigma with collapsed or nonfunctional papillae, which was observed in 1:1 herbicide-contaminated trial, is vulnerable to foreign particles.

Conclusion

Our results suggest that higher dust-to-pollen ratio, particularly dust-containing herbicides, can prevent successful pollination, resulting in poor fruit set and nut quality. To decrease the threat of dust interfering with pollination, the planting of windbreaks, particularly for young orchards where spring winds are a perennial problem, not mowing during pollination, and applying preemergent herbicides as early before pollination as possible are potential control measures.

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