University of California, Davis

Project Plan/Research Grant Proposal 2017 – Title and Summary Page

Project Title: Dust Influences Pollen Density and Pollination Quality in Pistachio

Project Leader:

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Co-PI(s)

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Project Year: 2017 (Year 1 of 2)

Anticipated Duration of project: 2 Years

Key words: pollination, pollen density, parthenocarpy, dust-pollination

Budget: <u>\$2,9995.00</u>

Summary:

Preliminary Study on the Influence of Dust on Pollination in Pistachio

In spring of 2009 a grower's observations of observations considerable dust generated by a flail mower, prompted a preliminary study on the of the the effect of dust on pollination and fruit set.

Bagged Kerman flowers were hand pollinated on three successive days with dust, pollen, dust and a pollen mixture (v:v=1:1) during bloom and a bagged control was maintained at the same time. The nuts per cluster were counted and weighed at harvest.

Nut set and production was extremely low in the clusters receiving only dust compared to clusters receiving only pollen and a dust: pollen mixture; those receiving dust produced 32% in nut count and 38% in nut weight, compared to clusters receiving pollen. Nut production in the pollen and dust mixture treatment was not significantly lower than that of clusters receiving pollen, suggesting that pollen was competitive in pollination above a certain threshold density.

These very preliminary results suggest orchard dust could interfere with pollination. We propose to conduct complete trial on the effect dust on pollination, and the effects of pollen density on pollination and fertilization, in fruit set and blanking in 2017.

Introduction and Rationale:

All the Pistacia vera cultivars, Kerman, Lost Hills, Golden Hills and Gumdrop, require pollination by their pollinizers, Peters and Randy, and fertilization to produce nuts. Pistachios are dioecious and anemophily species; pollen is carried from male to female trees by air currents. Both the male staminate and female pistillate inflorescences are panicles composed of hundreds of individual small flowers. The florets of both female and male flowers are apetalous with stigmas and anthers fully exposed to air and vulnerable to hostile environmental conditions such as wind, high or low heat and rain (Ferguson et al., 2005). The female flower consists of three parts stigma, style and ovary. The stigma functions as the pollen receptor and supplies exudates for pollen hydration and germination. In pistachio, the life-span of the stigma of an individual floret is 2-3 days; after that the surface of stigma senesces and cannot be pollinated (González et al., 1995; Hedhly et al., 2003.). Upon germinating, the pollen tubes grow length of the style female floret style reaching the micropyle, the opening in the ovary's outer layer, where it enters the ovary and completes fertilization. Successful fruit set is determined by stigma condition, pollen 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).

The combination of dry conditions and orchard floor management operations have been observed to generate considerable dust during pollination. The diameter of orchard dust is between 1-100 μ m while the diameter of female stigma and male pollen are approximately 500 μ m and 20 μ m, respectively. At these dimensions, dust can damage fruit set in two ways. First, dust could precipitate premature degeneration of female flower stigma through dessication. Second the dust could stimulate parthenocarpy; fruit set without fertilization as dust can stimulate the female flower to produce hormones for ovary expansion. However, because there is no pollen present to germinate and grow a pollen tube to the deliver the male germplasm to the ovary, the result is no fertilization and therefore no embryo to grow into a nut kernel. The result is either an aborted nut or an unfilled nutshell, a blank.

Our preliminary results research supported our initial prediction that field dust precipitated ineffective pollination. When dust was applied a few nuts were produced and the blank percentage was high, suggesting the dust caused parthenocarpy. Little information investigating how foreign material affects the stigma to produce parthenocarpy in pistachio. Our preliminary results demonstrated a 1:1 pollen:dust (v/v) mixture can successfully produce pollination and fertilization. As the natural pollen density range in California orchards is unknown, and dust is often produced by farm operations and windy weather we propose to study the threshold pollen density for successful fruit set in pistachio orchards.

This proposed research will focus on following objectives:

- 1. Investigate how dust affects pollination, focusing on the stigma structure and function, effective pollination period, movement of dust *in vivo* and pollination related hormone levels in flowers.
- 2. Test and suggest minimum, optimum and overload pollen densities for pistachio pollination.
- 3. Develop a method to measure and indicate orchard pollen density.

Methods and Materials:

Duplicate trials will be conducted in the field and laboratory.

<u>Pre-preparation</u>: In spring, pollen from male Peters pistachio trees will be collected in early blooming orchards. The pollen will be dried in room temperature for 3 days and pollen grain activity will be tested using the fluorescein diacetate staining (FDA) method.

Before bloom, clusters with flowers at then green extension stage will be isolated and covered with paper bags.

1.The Investment Study on Pollen Density and Fruit Set

In the field bagged clusters will be hand-pollinated with different densities of a dust: pollen mixture every 3 hours on five successive days. The dust will be pre-processed at 300 F* for one hour to inactivate pollen that could be in the dust. Pollen and dust volumes at 1:1, 1:2, 1:4, 1:6, 1:8, 1:16 v/v will be prepared and pollen densities confirmed by microscope. The bags will be removed after pollination is completed. At harvest fruit set, blanks and yield will be recorded in the field trial. The threshold minimum pollen density in pistachio are expected to be suggested based on this research.

2.The Mechanism that Dust Influences Pollination

At bloom, branches with bagged inflorescences will be brought to the lab. Pure dust, pollen, dust and the pollen mixtures will be applied to fresh stigmas to observe the stigma structures and activity with the scanning electron microscope (SEM). Post-pollinated flowers will be sampled to observe pollen tube growth and possible dust movement in vivo (in the pistil, style and ovary) by the aniline blue staining and observed with a fluorescence microscope equipped with a Digital Camera System. Twenty ovaries of flowers of each trial will be removed and prepared for gibberellin analysis by HPLC-MS.

3.Pollen Density Test in the Field

Multiple methods to test pollen density in the field will be investigated. One of the method is to use colored cards with sticky surface, which is similar to 'Yellow Sticky Traps' but with different colors. The cards will be installed in different positions of the canopies and then the pollen/dust densities will be counted and recorded. Optical microscope is needed in the field.

Then, according to the result of threshold pollen density based on the "The Investment Study on Pollen Density and Fruit Set" research, the pollination quality in the orchards will be evaluated.

4.Preliminary Research in 2017

Methods to improve pollination and fertilization success, such as additional Boron sprays and ethylene inhibitors (e.g. AVG, aminoethoxyvinylglycine), will be tested to strength the pollen tube growth and extend flower life-spans. Based on the results larger scale field experiment will be proposed and conducted in the second year.

References:

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Budget request:

1.	Salaries and benefits: Junior Specialist @ 50%. FTE + benefits	16,145.00
2.	50% Lab Assistant April – August .50 X 16.50/hour X 160 hours/month x 5 months	6,600.00
3.	Travel to Wasco Trial Site for 3 day/2 nights per trip. (\$150 gas +\$300 lodging+\$100 food)* 5 times	2750.00
4.	Field supplies – pollen test equipment, paper bags, coolers, pollen brush, tapes, labels	1000.00
5.	Lab charges – chemicals and supplies (e.g. centrifuge tubes, glass slides, formalin solution, plant tissue stain agents)	1500.00
	Lab charges – Equipment renting (SEM, HPLC-MS)	2000.00
	Total	2,9995.00

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Scope of Work

Dr. Lu Zhang:

Responsible for overall coordination of the project, supervising Junior Specialist Narges Mahvelati in executing applications, cluster sampling, harvest, data collection and analysis and writing final report.

Student Assistants (TBD) will assist under supervision of Dr. Lu Zhang

No External Contractors: