Factors Affecting the Efficacy of AF36 Improvement of the Biocontrol Agent and Monitoring Commercial Applications

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INTRODUCTION

Aflatoxins are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus* in several crops including pistachio. Pistachio is occasionally contaminated with aflatoxin, but this poses a high risk to international trade, due to strict regulations of aflatoxin contamination in food and feeds. Aflatoxin control in crops is difficult, and the only reliable method of control is using atoxigenic (unable to produce toxins) strains of *A. flavus* to displace or exclude the aflatoxin-producing strains of both *A. flavus* and *A. parasiticus*. Currently, the only atoxigenic strain registered for use in pistachios, in California, is *A. flavus* AF36. The *A. flavus* AF36 strain is naturally widespread in California, occurring in all of the major pistachio-growing counties (Doster et al., 2014), but at too low a rate to significantly reduce the aflatoxin contamination potential of the natural population of *Aspergillus*. However, when the biocontrol *Aspergillus flavus* AF36 is applied on the pistachio orchards, the rate of the atoxigenic strain AF36 is increased and is able to compete and displace the toxin-producing strains and consequently reduce the aflatoxin-producing potential of the population.

Experimental applications of the atoxigenic biocontrol agent *A. flavus* AF36, to reduce aflatoxin contamination in pistachios in California, started in 2002. The application of the AF36 product was successful in substantially increasing the proportion of the atoxigenic strain AF36 within the population of *A. flavus*/*A. parasiticus* fungi (Doster et al., 2014). In addition, the nuts from the orchards treated with the AF36 product did not have a higher incidence of kernel decay by *A. flavus* than nuts from untreated areas, suggesting that applying AF36 will not increase the number of moldy nuts. Nut samples from the orchards treated with the AF36 product were also less likely to be contaminated with aflatoxin than those from untreated orchards. Previously, the biocontrol was formulated in sterilized wheat inoculated with the atoxigenic strain. Currently, a new formulation consisting of roasted sorghum grain coated with the atoxigenic strain has been developed and approved for its use in California crop nuts, including pistachio, almond, and fig. The new formulation, called *Aspergillus flavus* AF36 Prevail®, has been successful in applications on cotton in Arizona, and we believe that it will also be successful in applications on pistachios in California. However, to better protect crops from becoming contaminated with aflatoxins, more research is required to optimize the application of the biocontrol and ensure more displacement of toxigenic strains. Information about optimum conditions for sporulation and dispersal of the biocontrol product can help facilitate the displacement of toxigenic strains and, ultimately, can reduce aflatoxin in pistachios.

RESULTS

The vertical mobility, measured by spore density of *A. flavus* AF36 was studied under field conditions in an orchard at the Kearney Agricultural Research and Extension Center. Spore traps, consisting of eight 9-cm Petri dishes with 50 ml *Aspergillus* Differentiation Agar medium (Fluka, AFPA) per plate, were placed at four different heights from 30 to 230 cm. The biocontrol product *A. flavus* AF36 Prevail® was applied on the soil under the spore traps. In general, results indicated that *A. flavus* spores decreased exponentially as a function of height, while the density of *A. niger* spores increased. Although further experiments need to be conducted, this situation could be explained by the fact that the tree canopy could work as a natural source of inoculum for *A. niger*. It is important to consider that *A. niger* does not produce aflatoxins, but some isolates can produce ochratoxins, another important concern for the nut industries in California.

To determine the best placement, with respect to irrigation, for maximizing the sporulation of the atoxigenic *A. flavus* biocontrol product, AF36 Prevail® grains were placed at different distances (from 25 to 250 cm) from the irrigation microsprinklers under field conditions. Both the sporulation of the *A. flavus* AF36 product grains and the soil water content were periodically evaluated. In this experiment, we observed that sporulation of the AF36 product grains was optimal where soil moisture was between 13 and 18 percent. Conversely, AF36 sporulation was practically nonexistent in soil where there was excess (> 24 percent water content) or limited amount (<6 percent water content) soil water content.

To determine the distance that the atoxigenic biocontrol fungus *A. flavus* AF36 is able to disperse from the source of inoculum in nut orchards, we applied the AF36 product, at 10 times the normal rate on the soil around one tree in the center of an orchard at Kearney Agricultural Research and Extension Center. Preliminary results indicate that the fungus is able to move in all directions and that the spore density of total *Aspergillus flavus* decreased exponentially with increased distance from the source.

The impact of different arthropods, as a potential cause of atoxigenic biocontrol product predation and loss, was evaluated under field conditions. A video camera (BirdCam, Wingscapes) was placed to monitor feeding behavior of arthropods on soils where the biocontrol product was applied. Results indicated that in nontilled soils, *Oniscidea* species (roly polies or pill bugs) and different ant species could feed on the sorghum grain applied onto the soil. Conversely, the impact of these arthropods is minimal in frequently tilled soils.

Additionally, we will continue to analyze pistachio library samples for aflatoxins, and evaluate the effect of commercial application of AF36 Prevail®. These samples originate from commercial blocks that have been part of AF36 biocontrol research efforts since 2008. Some of the blocks have been left untreated since the start of the project. Other blocks have been treated with 5 or 10 lbs. per acre of the product annually. Following harvest, samples are recovered from the Wonderful Orchards processing facility and, after sorting, they are ground and mixed up. Then the aflatoxins are extracted and run through special immunoaffinity columns, which bind the aflatoxin, and finally are run through high-performance liquid chromatography for aflatoxin detection. The results are analyzed to determine the effect of different rates of treatment with the *A. flavus* AF36 Prevail® product.

CONCLUSION AND APPLICATIONS

The results presented here will be useful to optimize application of the biocontrol product *Aspergillus flavus* AF36 Prevail® and to devise aflatoxin reduction management strategies in pistachio crops in California. These studies indicate that when conditions are optimal, the

product will readily sporulate and disperse. If the spores of the biocontrol strain are able to spread throughout the orchard and into the tree canopy, the potential to displace toxigenic isolates increases, while the probability of aflatoxin contamination decreases. Our findings indicate that it is important to consider product placement with respect to the irrigation source and to consider the potential for product loss due to arthropods. Future research will focus on application strategies to deliver the biocontrol agent to the orchard environment in a timely matter, even when conditions for sporulation of the product are suboptimal. New formulations and the optimal timing of application will be investigated. Also, we will look for other atoxigenic strains that can be developed that could have advantages in different environmental conditions.