Inoculum monitoring of biological control agent AF36, *Aspergillus flavus*, under field conditions

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COFUNDED BY MARIE CURIE ACTIONS

INTRODUCTION

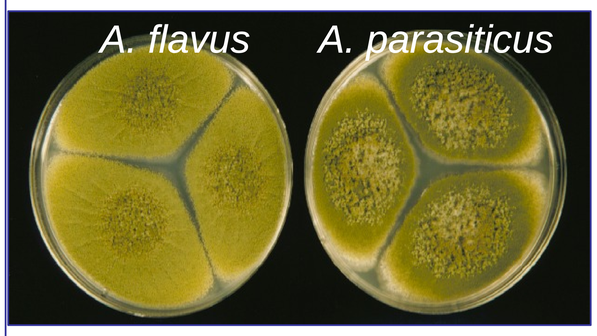

Figure 1. Fungi that can produce aflatoxins in almond orchards in California.

Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus* (Fig. 1), are among the most potent natural carcinogens and are widely regulated by governments who have set very low tolerances for aflatoxins in food and animal feeds. California nut crops and other crops such as fig can be contaminated with aflatoxins (Fig. 2). During the last decade, we have been working on reducing aflatoxin contamination of nuts and figs using the atoxigenic strain of *A. flavus* AF36. Fortunately, the biological control *A. flavus* AF36 Prevail® has been approved for use in pistachio (2012), almond (2017), and fig (2017) orchards in California by the Environmental Protection Agency (EPA) and the California Department of Pesticide Regulation. Here, we present different field studies aimed to understand the dynamic of the atoxigenic *A. flavus* AF36 inoculum under field conditions in California.

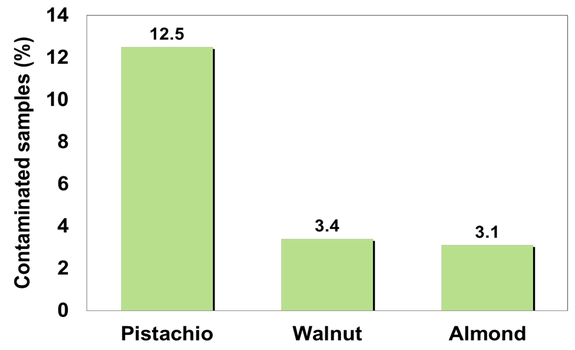

Figure 2. Californian nut samples contaminated with aflatoxins (data by the Dried Fruit Association).

PROCEDURES AND RESULTS

Figure 3. Spore trap placed in the field.

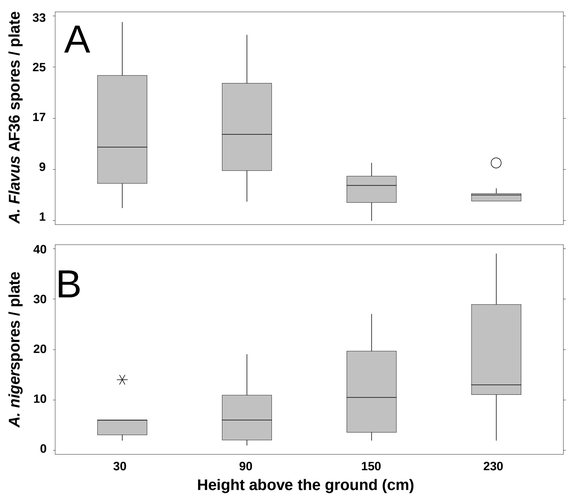

Figure 4. Box plots of **(A)** *Aspergillus flavus* AF36 and **(B)** *A. niger* spores per Petri dish at different heights above the ground (cm) where the biocontrol AF36 Prevail® was applied. The line within the box is the median. The top and bottom lines of the box represent 25 and 75th percentile of the data. Lines extending horizontally beyond the box represent the 5 and 95th percentiles.

Effect of height on A. flavus AF36 spore density., The effect of height above the ground on spores 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 different heights from 30 to 230 cm. The biocontrol product *A. flavus* AF36 Prevail® was applied on the soil under the spore traps (Fig. 3). In general, results indicate that *A. flavus* spores decreased exponentially as a function of height, while the density of *A. niger* spores increased (Fig. 4). 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 of its isolates can produce ochratoxins, another important concern for the nut industries in California.

Effect of soil water content on A. flavus AF36 sporulation. To determine the placement of the atoxigenic *A. flavus* biocontrol product grains for optimal sporulation, *A. flavus* AF36 Prevail® product grains were placed at different distances (from 25 to 250 cm) from the irrigation micro-sprinklers under field conditions. Both the density of sporulation of the *A. flavus* AF36 product grains and soil humidity by gravimetric analysis were periodically evaluated. In this experiment, we observed that there was an optimal sporulation of the AF36 product grains in the areas where soil moisture was between 13 and 18%. Conversely, AF36 sporulation was practically nonexistent in soil areas where there was excess (> 24% water content) or limited amount (<6% water content) of irrigation water (Fig. 5).

***Spore dispersal*.** 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 a rate 10 times the normal, 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 at all directions. However, the population of total *Aspergillus flavus* decreased exponentially as distance from the spore source increased (Fig. 6).

Impact of arthropods on AF36 inoculum. The impact of different arthropods as potential cause of atoxigenic biocontrol product 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 indicate that in no tilled soils, *Oniscidea* species (roly poly or pill bugs) and different ant species (Fig. 7) can impact the residence of the applied sorghum grain in the soil. Conversely, the impact of both arthropods is minimum in frequently tilled soil.

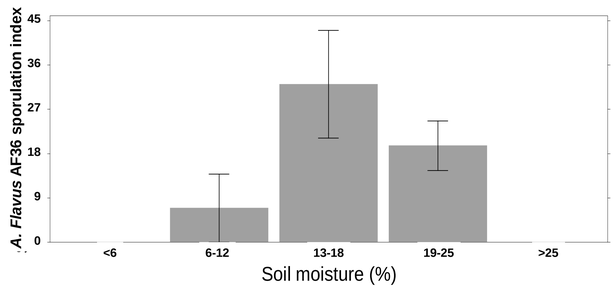

Figure 5. Effect of the soil moisture (%) on sporulation of *Aspergillus flavus* (AF36 Prevail®) biocontrol product grains.

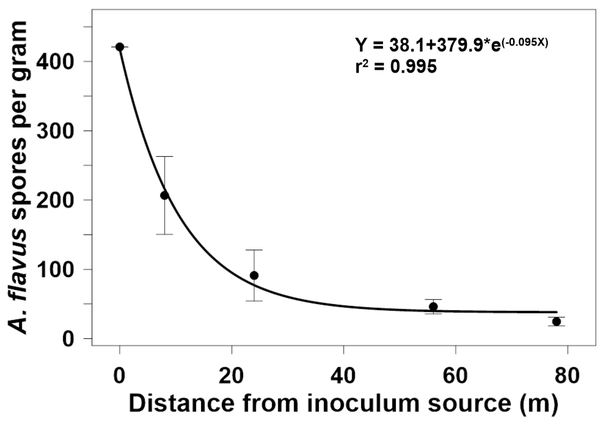

Figure 6. Number of spores of *Aspergillus flavus* per gram of leaf as a function of distance from the inoculum source.

Figure 7. Pill bugs and ants caught interacting with the biocontrol sorghum grains.

Acknowledgments

Almond Board of California (Project 17-PATH16-Michailides)

Juan Moral holds a Marie Skłodowska Curie fellowship launched by the European Union's H2020 (contract No 658579)

Teresa García holds a mobility scholarship launched by the government of Spain. ARGO GLOBAL program.