CHALLENGES IN ADAPTING IN PRACTICE THE NEW TRENDS OF AGRICULTURAL RESEARCH: «A PLANT PATHOLOGIST'S PROSPECTIVE»

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Summary

Plant pathology is a discipline that addresses the diagnosis of plant diseases and the increase of the fundamental understanding of host-pathogen interactions for the purpose of preventing or mitigating crop losses. For a number of decades, major agricultural colleges and universities worldwide used to have a Plant Pathology or a Crop Protection Department to educate students in the pest management discipline. However, in the last two decades, major socioeconomic changes have occurred that led the administrators of the majority of these institutions to implement contentious reorganization of the education and the specialties each university could provide to its students. Similar changes and challenges were also implemented by the University of California. For example, within the UC system the three campuses with Plant Pathology Departments were UC Berkeley, UC Davis, and UC Riverside. The Department of Plant Pathology at UC Berkeley was eliminated as a consequence of reorganization (1992 -94). The Department of Plant Pathology at UC Riverside was merged with the Department of Microbiology to become the Department of Plant Pathology and Microbiology. Only the Department of Plant Pathology at UC Davis maintained its integrity, but this one still had to be administered as a cluster together with the Department of Entomology and the Department of Nematology. Through this reorganization and the advances made in molecular science, there was a competition among universities in hiring top scientists to address molecular aspects of pathogens and diseases; while at the same time a significant shrinkage in the number of faculty members addressing problem-solving approaches has occurred. Changes in colleges and universities have placed a lot of pressure on the heads of departments to do "mission oriented" research. However, the mission oriented research was directed at major systems, resulting in diluting the structure that was essential to address the applied research and problem-solving approaches. As plant pathologists being in the midst of agricultural industries, we were faced with a big dilemma: how to reestablish the problem solving continuum where it has been broken and strengthen it where it has been weakened, and how to bring together scientists who are devoted to basic molecular research with those who try to solve field-problems for the agricultural industries. Plants are the source of life, and will always suffer by various known and emerging diseases. Challenges in the prognosis and understanding of these diseases, the study of the environmental conditions that affect the diseases, the changes in the populations and the interactions of pathogens and hosts, and the development of cost effective tools and management approaches are all tasks and responsibilities of plant pathologists now and in the future. This presentation will report a few examples showing the effective merging of the two aspects of research, such as basic use of molecular tools and approaches to be used in agricultural production in combating plant diseases. It is the philosophy of the authors of this report that laboratory and field research in agricultural commodities should go hand-in-hand with the ultimate goal to provide solutions to problems in the field in an efficient, sustainable, and cost-effective way so that growers achieve maximum revenues with low inputs and without affecting the environment.

Keywords: Aflatoxin, Alternaria late blight, atoxigenic strain, *Aspergillus flavus,* fungicide resistance, latent infection, biopesticide, pistachio

Introduction

Plant pathology is defined as the science addressing the diagnosis of plant diseases, the elucidation of plant pathogens and disease cycles, the understanding of the host-pathogen-environment interactions, and most importantly, the management of plant diseases. Over the years, universities have developed separate Departments, offered courses relevant to plant pathology, and granted degrees (M.S. and Ph.D.) in Plant Pathology, although these initial Departments were Dept. of Plant Pathology, Dept. of Crop Protection, or Dept. of Botany and Plant Pathology, etc.

The University of California has now 10 campuses located in strategic locations throughout California. Among these campuses, only three (Berkeley, Davis, Riverside) had a Dept. of Plant Pathology. One of the oldest departments in plant pathology, the Department of Plant Pathology at UC Berkeley, was closed in 1993 due to the re-organization of the college, leaving only UC Davis and UC Riverside now that offer graduate degrees in Plant Pathology from the Department of Plant Pathology at UC Davis and the Dept. of Plant Pathology and Microbiology at UC Riverside. Due to recent economic challenges, although the Dept. of Plant Pathology at UC Davis has maintained its integrity and its name, it is now administered as a cluster along with the Dept. of Nematology and Entomology. In any case, these and other departments in USA are active in educating and granting graduate degrees in Plant Pathology to national and international students.

Changes/challenges: In the last quarter of the twentieth century, there was a major shift in research due to advancement of molecular science which has revolutionized our understanding of biological processes and functions of physiological and biochemical properties of cells. This shift in research priorities has been also influenced by socio-economic changes which in turn affected the direction of research by the various universities worldwide. As a consequence, agricultural colleges set their mission and priorities of research and started hiring researchers using the criterion of "mission oriented research" but at the same time giving emphasis on narrowly focused individuals. By doing this there was a major competition among universities as whom is going to hire the best molecular biologists to teach, train, and do research to advance molecular biology even further at the expense of "practical research." Similarly, in departments of plant pathology, researchers started addressing molecular aspects of research of pathogens and emphasized the correct identification of the pathogen, phylogenetic and spatial relationships of pathogens, molecular aspects of genetic relationships, and also molecular ways in looking into pathogen-host interactions. Departments have refused to fill positions of individuals who were doing research that had immediate implementation (practical research). Unfortunately, this trend occurred during a relatively short time during which a large number of the "old fashioned" plant pathologists had retired. The situation became even worse when in the last decade or so, there was a major shortage of research funding, or the majority of the funds were directed towards more basic and molecular research and towards more research to "save the environment" than more problem-solving and applied research.

One of the oldest and most famous plant pathology departments of the USA, the Department of Plant Pathology at UC Berkeley closed its doors in 1993 with some faculty members retiring and the active ones re-assigned to other departments of the Berkeley campus or the Department of Plant Pathology at UC Davis. Furthermore, due to financial problems, faculty who retired were not replaced, and those still active undertook more responsibilities in their programs. Universities set five to ten years plans in addressing major issues instead of addressing short–term and/or specific programmatic missions. To prepare for the future that includes expansion in population, diminished access to water and prime agricultural land, and a changing climate that will require agricultural practices to adjust to altered temperature regimes and rainfall patterns and expansion in urbanization, the University of California has set major goals and took steps towards accomplishing these missions. These socioeconomic changes, the reduced research funding, the reorganization of university departments, have contributed tremendously in all these new trends in research in the last decade or so.

Shortage in funding by federal and State agencies due to large federal budget deficits, shrinkage in hiring faculty to address problem-solving research, shrinkage in hiring extension agents (farm advisors and specialists) to address mainly practical regional problems and provide solutions, all contributed to dismantle research units that raised challenges to the industry clientele who expect answers to their questions and solutions to challenging situations. Moreover, expectations and selection of criteria for the promotion and merit of farm advisors, specialists, and faculty have changed. All these individuals now are expected to carry research projects and spend time in writing refereed articles in addition to their day-to-day farm calls, outreach and extension work, and training of undergraduate and graduate students and postdoctoral research associates. The acreage of some specialty crops has increased (i.e. nut crops in California), new crops (i.e. pomegranate, blueberries, jujube, etc.) have been introduced and established lately, cultural practices have changed, and the expectations of agricultural business investors have increased. Information on cultural practices and pest management of these new crops is very limited to nonexistent and "expert" specialists do not exist. Despite these challenges, farm advisors, extension specialists, and academics made adjustments in their day-to-day work in order to satisfy on the one hand their administrators and on the other the industries clientele. University and industry funds have been reduced and the general feeling is to "do more with less." Although in some cases this approach has worked well, in most of the cases the industry clientele are frustrated by the fact that they do not get immediate attention and solution of their problems.

Two major challenges include a continuous competition and controversy between the interests of urban vs. rural communities. A third challenge is the exceedingly increasing demands of environmental groups for preserving natural habitats, water, and environment without weighing against agricultural land and production practices farmers use to produce all these wonderful products to place on the table of all the people. A fourth challenge, and this one is more programmatic, is that many researchers are now involved with research on global issues, leaving not many individuals that address local issues and solutions to smaller problems that provide answers to immediate questions by farmers.

More recently, however, federal research-funding agencies and universities took various steps and set priorities to address the above stated challenges. Some examples of recent priorities include a) satisfy urban populations /environmentalists since their priorities are very different from those of rural communities; b) alleviate the continuous competition of urban vs. agricultural communities for water; c) prevent the continuous invasion of exotic pests and diseases whose management is very difficult, particularly when these invasions are in the proximity of urban communities; and d) prevent contamination of food supply with microorganisms and/or mycotoxins (i.e. increase food safety).

There is a need for balanced research. This can be accomplished in the following ways: i) agricultural researchers need to focus locally while having in mind the global picture; ii) make research results relevant to agricultural communities; iii) there is a necessity to strengthen the relationship between researchers and farmer organizations; and iv) transfer the results from the laboratory to the field as soon as possible. Considering all these, the American Phytopathological Society has raised some concerns of great magnitude. For instance, there is a trend for losing field-oriented expertise in departments and for reduced federal funding to address field-oriented research (resulting in reduced funding to support students). Furthermore, there is a clear trend in applicants for various positions to have a narrow set of skills (MacDonald et al., 2009).

Fortunately, the University of California, as a land grant institution, has had a mandate to extend the knowledge from the laboratory to the field and the local community. Moreover, sometimes these systems can be used globally. The establishment of the Agricultural Research and Extension Centers at strategic locations is an indication of the strong interest of the University of California in the local agricultural communities. The largest among these centers is the Kearney Agricultural Research and Extension Center, which is located at Parlier, a small agricultural community, 40 Km south of Fresno.

The Kearney Agricultural Research and Extension Center is located in a strategic agricultural location, the center of the San Joaquin Valley where major agricultural crops are grown. Some representative crops include stone fruit, grapes, almonds, citrus, pistachios, walnuts, pomegranates, and persimmons. In addition, avocados are grown along the foothills of the Sierra Nevada Mountains. Our research has focused on studies of the epidemiology of tree and vine diseases and emphasized the development of simple techniques for the detection, prediction, and management of these diseases. And furthermore, whenever possible, we have used new technological advances to answer questions in disease epidemiology, prediction, and management. Below we describe three such examples where research that was developed in the laboratory was extended to the field and helped growers to make decisions in disease management.

Example 1. Diagnosis and monitoring of fungicide resistance by molecular techniques in phytopathogenic fungi.

Resistance to systemic and reduced risk fungicides has been a major challenge for crop protection since the early

1970s. The conventional method for checking resistance consists of making a random collection of isolates, single spore them, and then transfer single spore isolates to media amended with various concentrations of the fungicide, and measure mycelial growth or spore inhibition after a few days of incubation. Specifically, this example deals with the detection of azoxystrobin resistance in Alternaria late blight of pistachio and leaf spot of almond. Both these diseases are very devastating diseases of these nut crops with leaf spot disease of almond showing earlier in season and causing severe premature defoliation and late blight of pistachio showing later in season (after July) and becoming very severe during harvest. Severe Alternaria of pistachio at harvest can stain nuts and thus lower their quality and marketability. Management of these diseases relies heavily on preventive fungicides (2-4 applications/season) and specific cultural practices. Fungicides currently used for Alternaria diseases include solo products and various formulated mixtures of fungicides (Groups 3/11, 9/11, and 7/11). With the exception of chlorothalonil and iprodione, all the fungicides registered for the Alternaria diseases of pistachio and almond exhibit medium (mixtures) to high (solo products) resistance risk. One of the strobilurins, azoxystrobin, was registered for control of Alternaria diseases of nut crops in 2000. In only a few years after the registration of azoxystrobin, failures in the field were detected. And in 2003 resistance of *Alternaria alternata* to azoxystrobin was confirmed. In fact, the G143A mutated gene was associated with this resistance in pistachio and almond isolates of *Alternaria alternata* (Ma et al., 2003). When isolates resistant to azoxystrobin as determined with the conventional method of spore germination were compared with sensitive (wild isolates), only the resistant isolates had the codon 143 where the aminoacid glycine replaced alanine (G143A). None of the sensitive isolates had such a replacement (mutation). After the allele-specific primer pair ARF4 and ARR4 were shown to be very specific to the resistant isolates, an allele-specific PCR was developed to detect azoxystrobin resistance from cultures of the pathogen. The primer pair showed specificity in random samples of isolates of *Alternaria*. This high specificity was also applicable to samples of DNA extracted from diseased leaves infected by resistant isolates but was not specific to DNA extracted from diseased leaves infected with sensitive isolates. Since we had then a PCR technique to detect resistant isolates directly from plant material, we considered the question of how widespread was the azoxystrobin resistance in pistachio and almond orchards. Using a real time PCR machine and the specific pair of primers, we then were able to quantify the resistance in the field. Random samples of infected leaves were collected from 37 pistachio and 4 almond orchards. Additional samples were collected to determine the best number of lesions one would need to extract DNA in order to determine the best representative level of resistance detectable in a field. We checked 10, 25, 50, 75, and 100 individual lesions collected from random diseased leaves. This was done for samples collected from three different orchards. Although there were not major differences in the detection of A143 among the various size categories of samples, the samples of the 50 random lesions were sufficient for representing the populations of *Alternaria* resistant isolates. This analysis was performed then in 50 randomly collected infection lesions from random leaf samples of all the sampled orchards and a Table was constructed (Table 1) which shows the frequency of azoxystrobin resistant allele (A143) using the developed real time PCR assay. By using the values from Table 1 and the standard curves from the real time PCR, developed with known amounts of DNA of resistant isolates, one could easily then determine the frequency of resistant allele (resistant isolates of *Alternaria*) in a field. For example, orchard P33 had a frequency of 0.7885 of resistant allele A143, and using the standard curve in Figure 1, this orchard had a 71.7% frequency of *Alternaria* isolates resistant to azoxystrobin (Luo et al., 2007).

Table 1. Quantitative determination of frequency of azoxystrobin resistant allele A143 (FA) using a real-time PCR assay.

Orchard	Date of collection	County	Host	Mean FA	SD
A1	11 Aug 2005	Glenn	Almond	0.9910	0.0010
A2	5 Aug 2005	Glenn	Almond	0.9960	0.0005
A3	1 Aug 2005	Glenn	Almond	0.9990	0.0011
A4	5 Aug 2005	Butte	Almond	0.9998	0.0008
P1	28 July 2005	Glenn	Pistachio	0.9926	0.0028
P2	19 Aug 2005	Fresno	Pistachio	0.0603	0.0489
…	…	…	…	…	…
P32	6 Sept 2005	Madera	Pistachio	0.9899	0.0150
P33	6 Sept 2005	Madera	Pistachio	0.7885	0.0544
P34	6 Sept 2005	Madera	Pistachio	0.9926	0.0104
P35	6 Sept 2005	Madera	Pistachio	0.9874	0.0179
P36	8 Sept 2005	Butte	Pistachio	0.9915	0.0098
P37	8 Sept 2005	San Joaquin	Pistachio	1.0000	0.0000

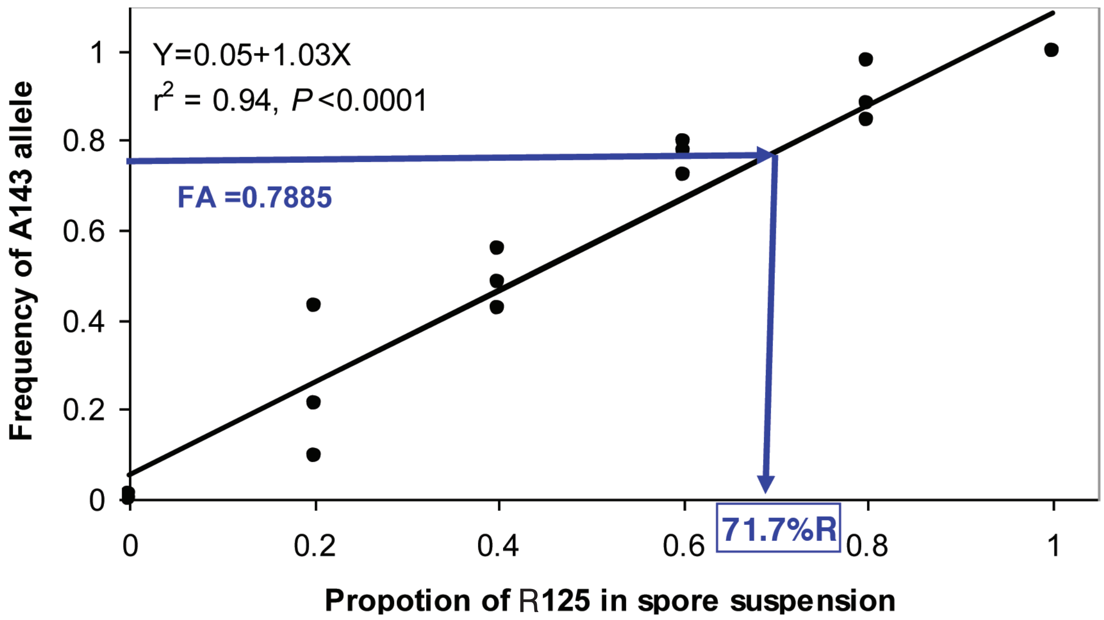
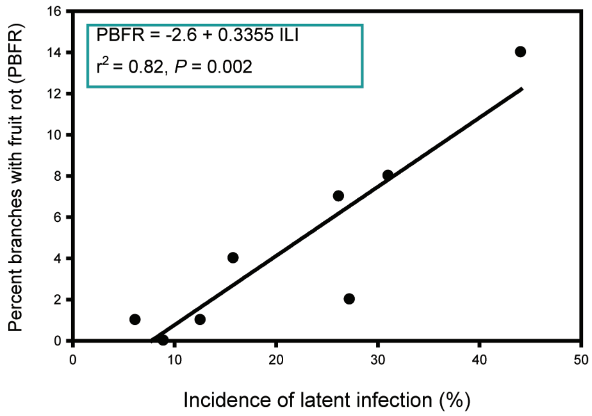


Figure 1. Linear relationship between the frequency of azoxystrobin-resistant allele A143 in *Alternaria* spp. (FA) and the corresponding proportions of azoxystrobin resistant (AR): azoxystrobin sensitive (AS) isolates (R125 = azoxystrobin-resistant isolate of *Alternaria*).

Example 2. Detection of latent infection of brown rot in stone fruit using PCR technology.

Brown rot caused by *Monilinia fructicola* and *Monilinia laxa* is considered as the most important disease of stone fruit in California, USA. When rains occur during the bloom of stone fruit, growers can suffer crop losses due to blossom and shoot blight. Therefore, preventative sprays are needed to protect blossoms and tender shoots from infection. Specifically, prune, also called dried plum (*Prunus domestica*), is very susceptible to brown rot and both species of brown rot fungi can attack prunes and cause significant damage. These pathogens infect green fruit and cause latent infections. Latent infections are defined as a parasitic phase of the pathogen that initiates and stops developing thus showing no macroscopic symptoms on the fruit (Sinclair & Cerkauskas, 1996). Our research has shown that in prune there is a strong correlation between the incidence of latent infections and the disease levels at harvest (Luo & Michailides, 2003). Initially, detecting latent infections using a conventional technique was very useful in predicting incidence of brown rot of fruit at harvest in the field. A technique that helped in determining such relationships between latent infection and brown rot levels was the overnight freezing – incubation technique (ONFIT). This method is now used for detecting latent infections of fungal pathogens in immature green fruit. The technique is simple, and relatively quick to perform, and very reliable. Fruit with viable latent infections will be covered with sporulation of the *Monilinia*, making them very distinct from other contaminant fungi.

Figure 2 shows an example of a strong correlation (r^2=0.82; P = 0.002). Using the ONFIT technique one can get an answer in 6 to 8 days after the date of collecting the fruit samples. However, waiting 6 to 8 days for an answer on disease levels, may create problems in managing the disease and/or more importantly, the timing for critical spray applications may have passed, leaving the grower helpless. The method we developed demonstrates how molecular methods can be used to get answers rapidly and provide timely answers to disease management questions. In plums (*Prunus salicina*) brown rot fungi can cause two types of infections of immature fruit: latent infections as defined above and quiescent infections. In contrast to latent infections, quiescent infections are macroscopically visible, although mycelial development is arrested after infection and resumes only as the host reaches maturity and/or senescence (Sinclair & Cerkauskas, 1996). Our question was whether molecular methodology could quantify latent and quiescent infections. To do this, samples of plums cv. Howard Sun were collected and separated into two sub-samples. One subsample consisted of fruit with minute brown/black specks (quiescent infections by *Monilinia*) and the second subsample consisted of fruit without any symptoms (assuming that putative latent infections were present) and the presence of *Monilinia* spp. were determined using two methods: a) ONFIT and b) PCR using specifically fruit-to-fruit contact surfaces of fruit skin where it was shown in previous studies that frequency of *Monilinia fructicola* infections are more common than in the remaining surface of fruit (Michailides & Morgan, 1997). For the sub-sample of fruit bearing quiescent infections, we also performed two methods: a) the direct plating of these infections after cutting very small tissues; and b) PCR by extracting the fungal DNA from a number of these infections. A question was raised regarding the association of the incidence of latent infection and postharvest rot of fruit. Using the PCR technique, 7.9% of the samples with invisible latent infections were positive for DNA of *M. fructicola,* and 6.7% of the fruit processed with ONFIT developed brown rot (Table 2). Similarly, as expected when visible quiescent infections were used, 60.5% were positive for *M. fructicola* with the PCR technique and 54.3% of those plated on APDA developed colonies of *M. fructicola.* Most importantly, the traditional techniques required 5 to 9 days while the PCR technique provided the results with 30 hours (Table 2). For more details consult Michailides et al. (2005).



Thresholds	
Latent (%)	**Postharvest rot (%)**
10.7	1
22.7	5
37.6	10

Figure 2. Correlation of branches with fruit rot in the field (PBFR) with incidence of latent infection (ILI) determined using the overnight freezing-incubation technique (ONFIT) and established thresholds for postharvest rot of French prune.

Table 2. Summary of comparisons of various techniques used to detect *Monilinia fructicola* in Howard Sun plums.

Technique	Latent infections	Quiescent infections	Required time
PCR	7.9 %	60.5 %	1 day* + 6 hours
ONFIT	6.7 %	---	7-9 days
Direct plating	---	54.3 %	5-7 days

** The time includes 1-day pre-incubation of samples at 24±2ºC.*

Example 3: The use of a biopesticide to control aflatoxin contamination in almond, pistachio, and walnuts.

Aflatoxins are secondary metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*. There are crops that are more frequently contaminated such as peanuts, corn, and cottonseed, and crops that are rarely contaminated such as tree nuts, figs, spices, and others. Among the nuts, the incidence of aflatoxin contamination is higher in pistachios than in almonds and walnuts. Both the aflatoxigenic species of *Aspergillus* mentioned above occur in nut crop orchards of California. Our work has focused on pistachio first and subsequently on almonds. Frequently, people considered that aflatoxin contamination is a postharvest problem. It can become a postharvest problem if the nuts were not dried sufficiently (i.e. approx 6% moisture) or stored improperly. Contamination of nuts with aflatoxin in California is typically a preharvest problem, resulting from infections by aflatoxigenic fungi that occur in the orchard either on navel orangeworm (NOW) infested nuts and/or early split nuts. Although a lot of these nuts will be removed during sorting due to special characteristics (suture staining, oily shell, frass protruding thru the split, dark shell staining, etc.), there are still contaminated nuts that will escape the sorting and end up in the marketable product. Early split nuts and NOW-infested nuts are considered the main source of contamination of pistachios with aflatoxins. In fact, early split nuts (ES) are the preferred site where NOW oviposits. Early splits start appearing in the orchard in July and continue forming until harvest; they typically account for 2 to 5% of the crop. Although the industry has placed a lot of effort in removing during processing ES and NOW-infested nuts based on specific characteristics that these nuts exhibit, many contaminated nuts still escape the sorting process including some nuts that do not show any distinguishing features. Therefore, the California pistachio Industry has focused on finding ways to reduce aflatoxin contamination of nuts while they are still in the field.

The concept of using atoxigenic *A. flavus* strains to reduce aflatoxin was developed initially by Dr. P. Cotty, a USDA scientist in Arizona, who selected and studied the atoxigenic strain (AF36), which was initially registered on cottonseed. Following this line of research, another selected atoxigenic strain was registered on peanuts with the trade name afla-guard®. Subsequently, the AF36 strain was registered on corn. Once we discovered that the AF36 strain was the dominant strain encountered in California pistachio, almond, walnut, and fig orchards, we initiated research in micro-plots to obtain efficacy data on the Californian AF36 strain displacing the toxigenic *A. flavus* and *A. parasiticus* strains. This strain is inoculated on sterilized wheat seed that is applied on the orchard floor during mid June to mid July. When the wheat gets wet after orchard irrigation, the *A. flavus* is activated, grows, and covers the major part of wheat surface with sporulation. In this way, the atoxigenic strain produces huge amounts of spores, thus displacing the toxigenic strain of *A. flavus.* These displacement data were submitted to the EPA along with a request for an Experimental Use Permit (EUP) to treat 3,000 acres of commercial pistachios without a need for crop destruction. The EUP was approved in May 2007, and the first application of 10 lbs per acre (12.1 kg/ha) was done in the summer of 2008. The single application was also repeated in the summer of each 2009 and 2010 using the same treated and untreated (control) orchards for all three years. The application of the wheat inoculum was done using a four-wheeler vehicle. The ant bait spreaders in the rear of this vehicle has been adjusted to distribute the wheat inoculum. In addition to soil samples collected from replicated orchards, "library" nut samples were collected and analyzed for aflatoxins. Library samples consist of 20 pounds (approximately 9 kg) of fresh nuts taken at the processing plant as the harvested nuts are being unloaded. Each library sample represents approximately 50,000 lbs (22.5 tons) of harvested nuts. These samples are used by the processor to determine how much growers will be paid. In general, we showed a reduction of 23%, 39%, and 45% in the incidence of aflatoxin contamination in 2008, 2009, and 2010, respectively (Figure 3). When maturity of nuts is uneven, pistachio trees are harvested twice. The risk for aflatoxin contamination is higher for the second harvest pistachios due to higher levels of NOW infestation and the nuts being exposed to the orchard environment longer. Although there were only a few samples for 2008, the reduction of aflatoxin contaminated nuts for 2009 and 2010 was 24% and 85%, respectively (Figure 3). Based on these positive results on the efficacy of AF36 strain, the strain was registered as a biopesticide on pistachio in February 2012. Growers obtained enough AF36 inoculum of wheat and treated about 30,000 ha in 2012.

Pistachio growers in California routinely follow good agricultural practices (for example, never collecting nuts that fall onto the ground), have intensive management of NOW, and for the first time starting in 2012 they can now use the AF36 biopesticide to reduce aflatoxin contamination.

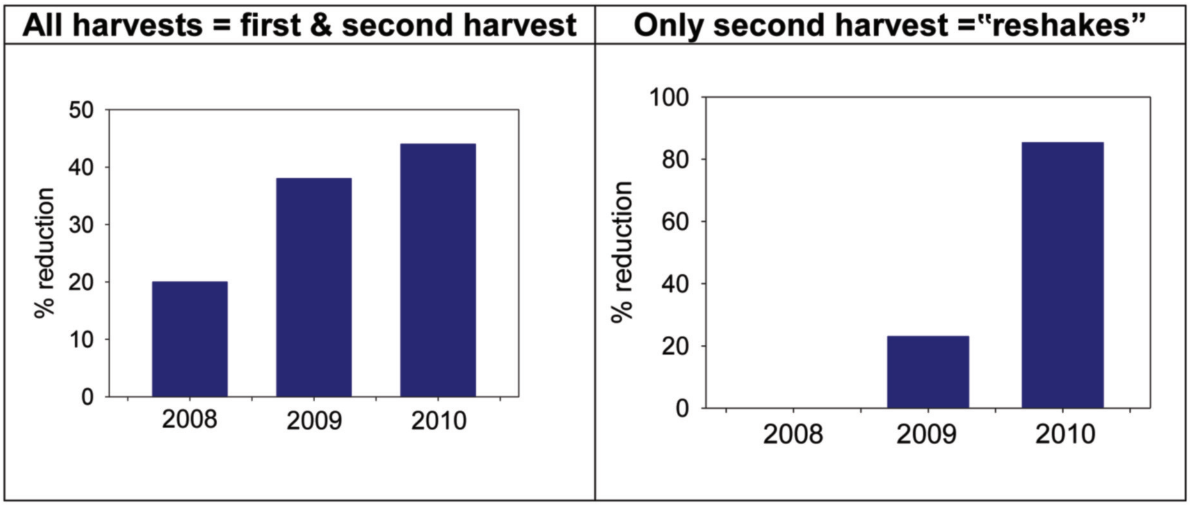


Figure 3. Percent reduction of aflatoxin contaminated pistachio samples after treating orchards with AF36 for three years.

Conclusions

The three examples of research presented here represent cases in which each completed project resulted in something that can be used by farmers and their consultants. For instance, the technique we developed in the laboratory with isolates of the *Alternaria* pathogen can be used by private laboratories to detect levels of resistance in populations of this pathogen in pistachio and almond orchards. With this knowledge, the grower can implement correct resistance management strategies for Alternaria late blight of pistachio and leaf spot of almond. In the second example, detecting and quantifying the latent infections of prune and plum fruit helps growers decide whether one or two preharvest sprays are necessary or not. Finally in the third example, research initiated in microplots of an experimental pistachio orchard was transferred to the field (initially 1,200 ha of pistachios) under an experimental use permit and resulted in a 45% reduction of aflatoxin contaminated pistachio samples. These results led to the federal and state registration of a biopesticide that growers can now use in their orchards. In fact, almost 30,000 ha of pistachio orchards have been treated in 2012 with the biopesticide, atoxigenic *Aspergillus flavus* strain AF36.

The three presented examples support my co-workers' and my research philosophy which goes like this: "Laboratory and field research in agricultural commodities should go hand-in-hand with an ultimate goal to provide solutions to problems in the field in an efficient sustainable, and cost-effective way so that growers achieve maximum revenues with low inputs and without affecting the environment."

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References

Luo, Y., and Michailides T.J. 2003. Threshold conditions that lead latent infection to prune fruit rot caused by *Monilinia fructicola.* Phytopathology 93:102-111.

Luo, Y., Ma, Z., Reyes, H. C., Morgan, D. P., and Michailides, T.J. 2007. Using real-time PCR to survey frequency of azoxystrobin-resistant allele G143A in *Alternaria* populations from almond and pistachio orchards in California. Pest Biochemistry & Physiology 88:328-336.

MacDonald, J. Allen, C., Gadoury, D., Jacobi, W., Kelemu, S., Moyer, J., Murray, T., Ong, K., Pearson, C., Sherwood, J. and Vidaver, A. 2009. Education in plant pathology: Present status and future challenges. Plant Disease 93: 1238-1251.

Ma, Z., Felts, D., and Michailides, T.J. 2003. Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. Pest. Biochemistry and Physiology 77:66-74.

Michailides, T.J., and Morgan, D. P. 1997. Influence of fruit-to-fruit contact on the susceptibility of French prune to infection by *Monilinia fructicola.* Plant Disease 81:1416-1424.

Michailides, T.J., Morgan, D. P., Ma, Z., Luo, Y., Felts, D., Doster, M. A., and Reyes, H. 2005. Conventional and molecular assays aid diagnosis of crop diseases and fungicide resistance. California Agriculture 59:115-123.

Sinclair, J.B. and Cerkauskas, R.F. 1996. Latent infection vs. endophytic colonization by fungi. Pages. 3-29 in: (Endophytic Fungi in Grasses and Woody Plants. Systematics, Ecology and Evolution. S. C. Redlin and L.M. Carris, eds. American Phytopathological Society, St. Paul, Minnesota.)