Fungicide resistance

Why it happens and how it may affect you

By Megan Dewdney

Fungicides have been used in agriculture for more than 200 years. The first fungicides were broad spectrum inorganic compounds with many target sites within a fungal cell. Examples of these still in use around the world are sulphur or copper salts like Bordeaux mixture. Many old classes of fungicides are also still in use and give effective control. Since many of these fungicides act on many target sites, little to no resistance has been found to these multisite fungicides.

As fungicides have become more focused on specific targets, they are more vulnerable to resistance development. This has been especially true of new generations of "softer" fungicides.

So what is fungicide resistance? Broadly, fungicide resistance is when a population of fungi is no longer sensitive enough to a particular fungicide to provide disease control. It occurs in response to the repeated use of a fungicide or a class of fungicides and is often referred to as field resistance.

Fungicide resistance is usually divided into two types: qualitative and quantitative resistance. A sudden and marked loss of control suggests qualitative resistance. If qualitative, there are two distinct populations in a location — one susceptible to the fungicide and the other resistant. Generally this type of resistance is stable.

Quantitative resistance appears gradually with diminished fungicide efficacy over time because the fungal population becomes less sensitive with repeated use. In this case, resistance can often be reversed as the population of fungi tends to become more sensitive to the fungicide after a period of disuse. The fungicide can then be incorporated back into spray programs as long as it is used less often and rotated carefully with products with alternate modes of action.

Fungicide resistance can seem to come out of nowhere. In actuality, a minute proportion of the population, on the order of 1:100 million individuals, carry a modified version of the gene that allows the fungus to survive a given fungicide. This is even before the fungicide is introduced. The modified gene happens to make that individual resistant to the fungicide in question by random chance. Without the fungicide, the modified gene does not give the individual a growth, reproduction or survival advantage compared to the individuals without the mutation. This mutation may even be harmful to the individual. Furthermore, the mutation could spontaneously disappear and reappear several times before the introduction of the fungicide.

Once the fungicide is introduced, the importance of the mutation changes. The fungicide selects for individuals that carry the mutant gene from the original population that now have an advantage over the rest of the population. Out of the individuals that survive the fungicide application, there is an increase in the proportion of resistant individuals in the population because of their better ability to survive and reproduce than sensitive individuals. This occurs every time a fungicide application is made and the proportion of resistant individuals slowly increases in the population until the population is no longer susceptible. Paradoxically, if a fungicide is highly effective, resistance development can be more rapid as the selection pressure for resistant individuals is higher. If a fungicide is only 80 percent effective, the buildup of resistant individuals will be slowed.

IMPORTANCE IN CITRUS PRODUCTION

So why is fungicide resistance important in citrus production now? Currently, it is of special concern to tangerine and tangerine hybrid growers for Alternaria brown spot (ABS) caused by *Alternaria alternata*. During the last two summers, we have visited a number of groves with ABS control failure after applications of a strobilurin fungicide (Abound®, Headline® or Gem®). Since qualitative resistance has been problematic with strobilurins in other commodities, it was one of the first things we explored after ruling out other factors.

Strobilurins are derived from the secondary metabolites of a small mushroom forming fungus, *Strobilurus tenacellus*. They are active against a wide range of fungi and have low toxicity for most non-target organisms with the exception of aquatic animals. Strobilurins inhibit mitochondrial respiration which generates energy for biological processes. When the mitochondria are blocked, fungi are not able to produce enough energy and subsequently die, especially during critical phases such as spore germination. Within the mitochondrion, the target is very specifically blocked. However, as mentioned above, there is natural variation in fungal populations and a small number of individuals have a different form of the target which is not blocked by the fungicide. These individuals are able to survive strobilurin applications, and in the case of ABS, form a larger proportion of the *Alternaria* population with each application until disease control fails.

Strobilurin resistance management

1. Make no more than two sequential applications of any strobilurin FRAC group 11 (Abound, Gem or Headline) without alternating to another fungicide with a different mode of action, currently Ferbam and copper. To be particularly careful to conserve strobilurin efficacy, it is recommended to rotate modes of action with each application.
2. Do not make more than four applications of all strobilurins combined in a year for all uses and never exceed the amount per acre per year (Abound 2.08 F 92.3 fl. oz./acre/season; Gem 25 WG 32 oz./acre/season; Gem 500 SC 15.2 fl. oz/acre/season; Headline 54 fl. oz./acre/season).
3. Control should begin before disease development and continue as indicated by recommended disease management practices. For guidance, consult the Citrus Pest Management Guide (http://www.crec.ifas.ufl.edu/extension/pest/index.htm).
4. Do not apply strobilurins in nurseries. Application of strobilurin in nurseries can result in selection of resistant strains which are then distributed on nursery stock to groves.

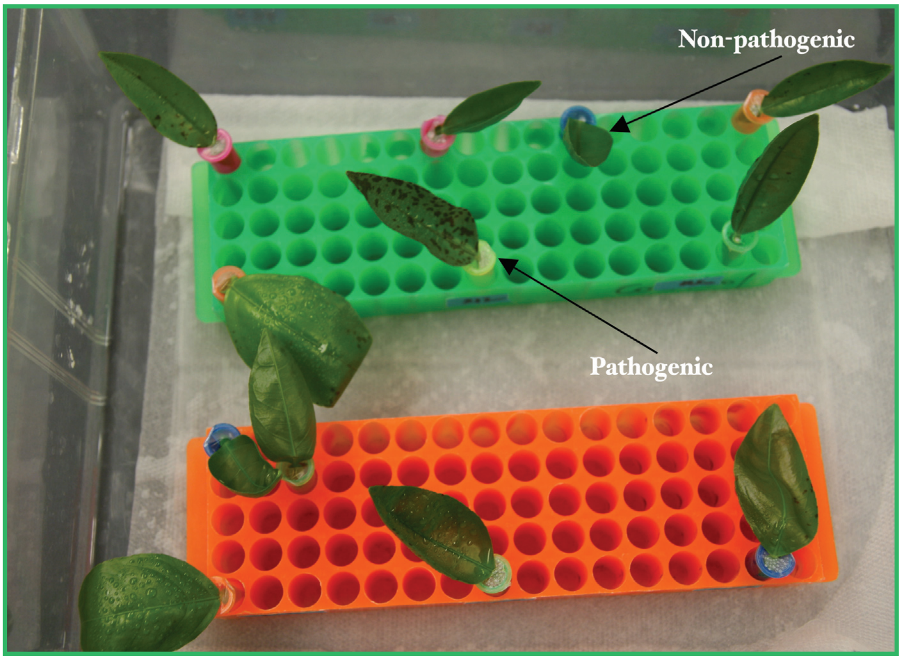

Figure 1. Minneola leaves inoculated with Alternaria spp. to determine whether it is A. alternata.

With great foresight, L.W. "Pete" Timmer (former University of Florida plant pathologist) undertook a baseline study before the introduction of strobilurin fungicides into the citrus market. He evaluated how sensitive fungal pathogens were to this fungicide class and gave an estimate of the native level of resistant individuals in a population. The information from such studies can be used to monitor for resistant populations once a fungicide has been in use as the strobilurins have been for *A. alternata*. In Timmer's study, it was found that *A. alternata* was the least sensitive of the citrus fungal pathogens to strobilurin fungicides.

Fungicide screening for *A. alternata* can be laborious. Once we have isolated *Alternaria* spp. from citrus leaves, each isolate needs to be screened to verify that it is pathogenic to citrus. Young leaves, usually from Minneola, are inoculated with a spore suspension and are incubated in a moist chamber until symptoms develop in two to three days (Fig. 1). This sometimes is slow because young flush is not always available. Once an isolate has been verified that it is *A. alternata* from leaf symptoms, a single spore is selected to start an isolate. This is to ensure that we are working with a genetically homogeneous isolate when we do the fungicide screening. After a week of growth, an isolate will be grown enough for us to initiate spore production and we can start the fungicide screening.

To determine the effective dose (ED) of a fungicide that kills 50 percent of the spores (termed ED_{50}) we add 10, 1, 0.1, 0.01, 0.001 and 0 ppm (parts per million) of the fungicide of interest — in our case Headline (pyraclostrobin) or Abound (azoxystrobin) — to artificial media. Gem was not tested, but we expect the results to be similar. In addition to the fungicides, a chemical know as SHAM is added to block the alternative respiratory pathway. This pathway allows fungi to produce a small amount of energy for a short period of time, but not enough to infect a plant. The results of the test can be inaccurate if SHAM is not used. For each isolate, the test is repeated at least twice. We place a known concentration of spores onto the amended media, wait overnight and then count the number of germinated spores out of a total of 50 spores for each fungicide concentration. From the range of fungicide concentrations, we then calculate the ED_{50} for each isolate.

In our studies we included some of the original baseline isolates (never exposed) collected by Pete Timmer to compare with the isolates we recently collected. All of the isolates collected by Timmer had ED_{50}s below 0.1 ppm of fungicide, indicating that they were sensitive to both Abound and Headline (Fig. 2). Most isolates collected in 2008-2009 from tangerine and tangerine hybrid groves from around the state with control failure had ED_{50}s >10 ppm of fungicide. This means that the isolates were insensitive or resistant to strobilurins. These results were confirmed by Gilberto Olaya of Syngenta Crop Protection. Any isolates that were susceptible to strobilurins were in the same range as the baseline isolates. The presence of two distinct populations with few intermediates is an example of qualitative resistance where isolates are either sensitive or highly resistant.

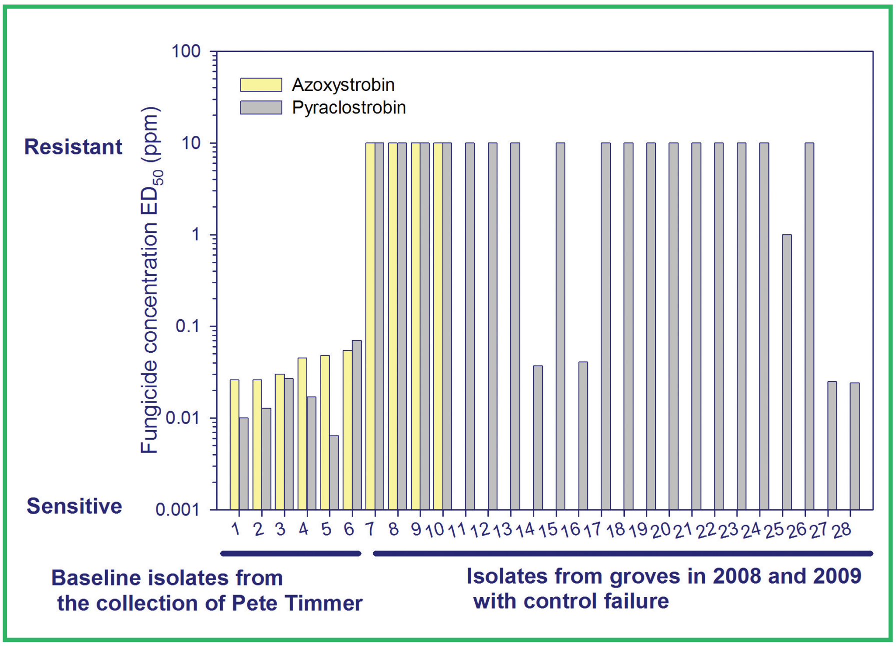

Figure 2. The ED_{50}s (fungicide dose that effectively kills 50 percent of the fungal spores) from selected baseline isolates and isolates in 2008-2009 collected from groves with suspected control failure. All Alternaria alternata isolates were tested for sensitivity to Abound® (azoxystrobin), but only some to Headline® (pyraclostrobin). All baseline isolates have ED_{50}s below 0.1 ppm of the fungicides indicating that they are sensitive. The ED_{50}s of most recent isolates are above 10 ppm indicating that they are resistant.

IMPORTANT FOR THE TANGERINE INDUSTRY

These results are important for the tangerine industry in Florida. If strobilurin resistance becomes widespread in the *A. alternata* population, it will be difficult to produce marketable tangerines and tangerine hybrids in Florida. It is clear that alternate modes of action are needed for rotation since copper and ferbam are the only other registered products for ABS control. We have been working with various companies to test new products for efficacy, but none have completed the registration process to date. Even with the introduction of fungicides with new modes of action for citrus foliar pathogen control, it will be important to follow careful resistance management strategies (see side bar) to preserve strobilurin efficacy where possible, but also make sure that resistance will not become problematic with future fungicides.

We plan to continue this project over the next few years. We are going to survey tangerine and tangerine hybrids throughout the entire state for strobilurin resistance to determine whether the problem is widespread. Currently, we are developing a method to test isolates more quickly than described here. Anyone interested in having their groves surveyed, please contact Megan Dewdney (mmdewdney@ufl.edu). We are also planning to confirm the mutations in the *A. alternata* isolates to ensure that our assumptions of which genes have been mutated are correct. The type of mutation present could change how fungicide resistance is managed.

CONCLUSION

In conclusion, cases of ABS control failure by spray programs that include strobilurin fungicides have been documented. Strobilurin resistance was identified as the cause of failure in all cases tested. Fungicide resistance should become a concern of tangerine and tangerine hybrid producers, especially those who sell mainly to the fresh market. More instances are very likely to be found around the state in the next few years. This is potentially a serious problem for growers as there are not many other effective fungicides registered for ABS control. In addition, other citrus fungal pathogens may also develop resistance in the future, so this is not a problem limited to tangerine and tangerine hybrid producers.

The author would like to thank Syngenta Crop Protection for material support for the survey project.

Megan Dewdney is assistant professor at the University of Florida's Citrus Research and Education Center, Lake Alfred.