Status of resistance of alternaria to fungicides in tangerine production

By Megan Dewdney and Byron Vega

Fungicides have been a mainstay of alternaria brown spot (ABS) control on tangerines since the discovery of the disease in Florida nearly 40 years ago. Over the years, there have been changes to the types of fungicides registered; the multisite, non-specific fungicides are less common and more fungicides with highly specific targets are available.

Multisite fungicides are still available and the most commonly used in citrus of these are copper compounds. The newer generation of fungicides have fewer effects on non-target organisms, but are also more vulnerable to resistance development.

So what is fungicide resistance? In general terms, fungicide resistance is when a population of fungi is no longer sensitive enough to a particular fungicide for the fungicide to provide disease control. It occurs when there has been repeated use of a fungicide or a class of fungicides, and is often referred to as field resistance.

QUALITATIVE AND QUANTITATIVE 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: one sensitive to the fungicide and the other resistant. This type of resistance is usually stable and does not revert back to a susceptible population.

Quantitative resistance appears gradually with diminished fungicide efficacy over time because the fungal population becomes less sensitive with repeated use. Quantitative 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 infrequently and carefully rotated with products with alternate modes of action.

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

Once the fungicide is introduced, the importance of the modification changes. The fungicide selects for individuals that carry the modified gene or genes from the original population to now give a survival advantage over the rest of the population. Of the individuals that survive the fungicide application, an increased proportion of the individuals in the population is resistant 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 sensitive. 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.

STROBILURINS

Over the last four years, for *Alternaria alternata*, the fungus that causes ABS, fungicide resistance has become a progressively more important problem for growers of tangerine and tangerine hybrids with increased reports of control failure after use of strobilurin fungicides (Abound®, Headline® or Gem®). In 2008 and 2009, we confirmed that qualitative fungicide resistance was the cause of control failure in some groves. Since the strobilurins are widely used in the industry and have been the only target-specific fungicide available for several years, we initiated a statewide survey to determine the strobilurin sensitivity in as many groves as possible. The survey is ongoing, and this is a report of our current findings. While the most pressing problem is with ABS, resistance can occur with the other fungal pathogens if the fungicides are not used with the proper precautions. The same principles of mode of action rotation apply to these other fungal diseases as well.

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 very specifically 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. However, as mentioned above, there is natural variation in fungal populations and a small number of individuals have a different target shape — mitochondrial respiration — that 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.

Before the 2002 introduction of strobilurin fungicides, L.W. "Pete" Timmer collected *A. alternata* isolates and we were able to establish the sensitivity of the native population. This baseline sensitivity allowed us to determine if any shifts in population sensitivity had occurred after the use of strobilurins. The baseline population was sensitive to Abound (azoxystrobin), and the effective concentration to inhibit fungal growth by 50 percent (EC_{50}) ranged between 0.01 and 1 ppm with 0.125 ppm as the most frequent value. The results with Headline (pyraclostrobin) were similar, although the fungus was slightly more sensitive to Headline.

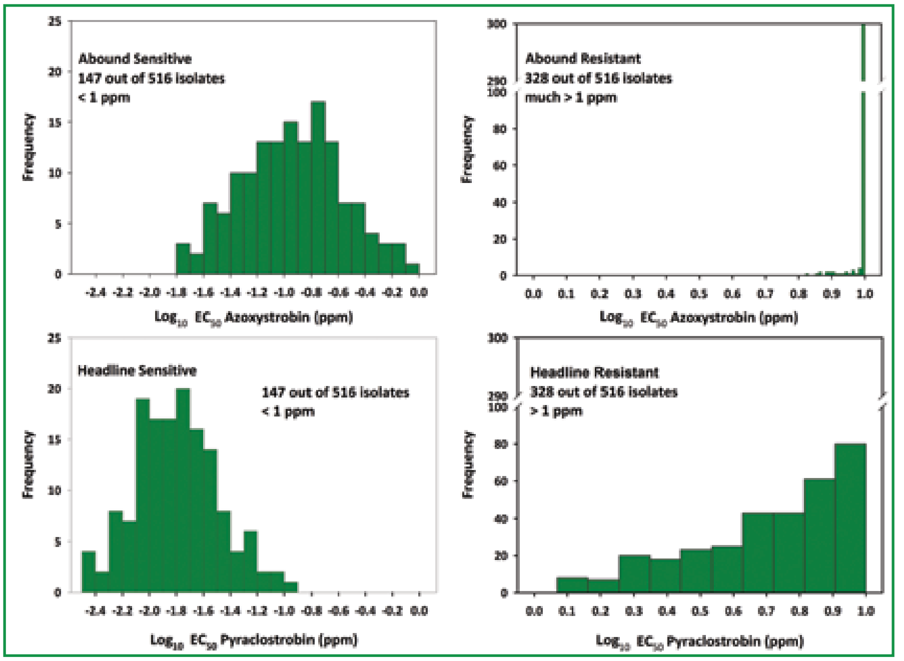

***Fig. 1.** The distribution of log_{10} EC_{50} values (effective concentration to inhibit fungal growth in 50 percent of isolates) for Abound and Headline of sensitive and resistant isolates collected in 2010 and 2011 in counties producing tangerines and their hybrids. EC_{50} values above 1 ppm of fungicide (log_{10} 0) would be considered resistant to strobilurins. We do not test at concentrations higher than 10 ppm (log_{10} 1.0) because this is more than would be normally found on a citrus tree.*

Of the 516 isolates collected in 2010-2011, 70 percent of the isolates collected from nine counties were found to be resistant to strobilurins. The EC_{50} levels of the sensitive population mirrored the range of the baseline population ($P > 0.05$), but the resistant population had EC_{50} values that were significantly higher than the baseline population ($P < 0.0001$) (Fig. 1). When tested with Abound, nearly all resistant isolates had an EC_{50} greater than 10 (log_{10} 1) ppm, which is more than the fungus would be exposed to in the grove (Fig. 1). The results with Headline were not as dramatic, but the EC_{50} values were greatly shifted toward higher concentrations of the fungicide (Fig. 1) as would be expected in a resistant population. The difference between the two fungicides shows that *A. alternata* is a little more sensitive to pyraclostrobin than to azoxystrobin.

Not all counties where isolates were collected in 2010-2011 had the same distribution of resistance. Polk and Osceola counties had the least, with between 50 percent and 70 percent of the isolates confirmed as resistant. The remaining counties where isolates were collected and tested (Lake, Seminole, Indian River, St. Lucie, Highlands, Hardee and Sarasota) had greater than 70 percent resistant isolates, meaning that most groves sampled in these areas had predominantly resistant populations (Fig. 2, page 17).

We were also interested to know if cultivar susceptibility had an influence on the percent of resistant *A. alternata* isolates. As it turned out, cultivar did have a significant influence on the percentage of isolates with resistance ($P < 0.0001$). Nearly 90 percent of the isolates from the highly susceptible Minneola were resistant. Similarly, between 65 percent to 75 percent of isolates from Dancy and Orlando were resistant, although the sample size was much smaller, so we are less confident in this result. Murcott had approximately 50 percent resistant isolates and the least susceptible cultivar, Sunburst, has only 30 percent resistant isolates (Fig. 3, page 17). There does appear to

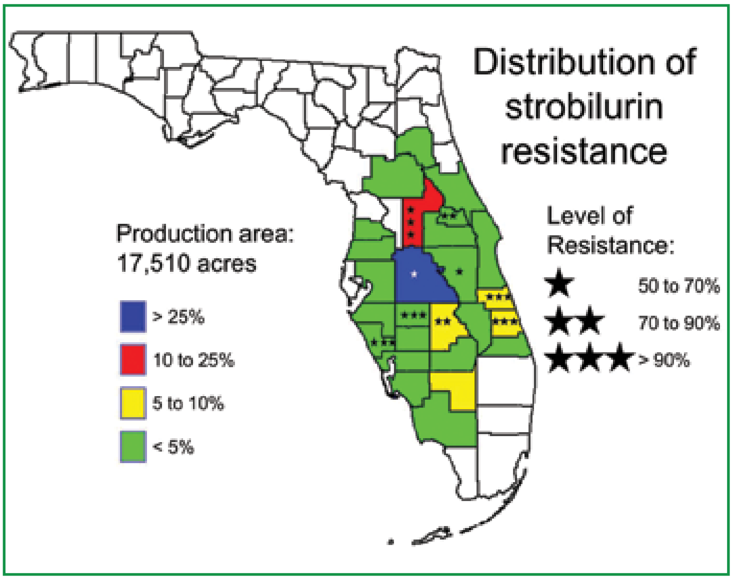

Fig. 2. The distribution of tangerine and tangerine hybrid production in 2011 and the proportion of strobilurin resistant isolates of Alternaria alternata found in each sampled county

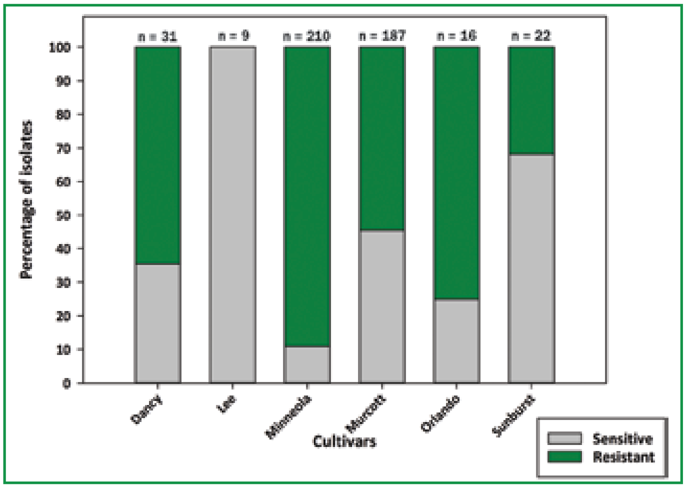

Fig. 3. The percentage of strobilurin resistant and sensitive isolates of Alternaria alternata sampled from tangerine and tangerine hybrid cultivars ("n" is the number of individuals that make up the sample per cultivar. It indicates how reliable the estimate of the total population is, with larger numbers being more reliable because there are more individuals that make up the estimate.)

be an effect of cultivar susceptibility on resistance development, and it is likely related to the number of applications needed to keep the fruit clean on highly susceptible cultivars and the value of the crop. Strobilurins are more likely to be used on cultivars of the greatest value.

There is some good news for tangerine and tangerine hybrid producers. Recently, alternate chemistries have been introduced to the citrus market. However like the strobilurins, the new chemistries are prone to resistance development and need to be used in rotation with other products from a different mode of action class. To assist with rotation, the modes of action are listed in the Florida Citrus Pest Management Guide and are labeled as FRAC (fungicide resistance action committee) codes. If the FRAC codes are the same values, then they are the same mode of action and resistance management recommendations should be observed, especially rotation. Pristine® and Quadris Top® are the two new products available for ABS management. Both products are pre-mixes with strobilurin fungicides, but the other compounds have different modes of action. Quadris Top is a mixture of azoxystrobin (FRAC 11) and difenoconazole (FRAC 3), whereas Pristine contains pyraclostrobin (FRAC 11) and boscalid (FRAC 7).

Strobilurin resistance has become a major issue for producers of tangerines and their hybrids in Florida. In our survey in 2010 and 2011, we found that the majority of A. alternata isolates were resistant to the strobilurin. We also found that there was a greater proportion of resistant isolates on highly susceptible cultivars. The survey is continuing in 2012, especially in areas that were under-represented to date, such as Lake County. Anyone interested in having their groves surveyed, contact Megan Dewdney (mmdewdney@ufl.edu).

We are now recommending that all growers manage their groves as if resistance were present to avoid costly control failures. This would include the incorporation of the recently introduced Pristine and Quadris Top into a program such as the example given in the accompanying sidebar below.

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Fungicide program for tangerines with strobilurin resistance

1. Should use pre-mixed fungicides with alternative modes of action such as Pristine or Quadris Top
2. Frequent rotation with copper or other multisite fungicides like ferbam
3. As general good practice, make no more than two sequential applications of any strobilurin FRAC group 11 (Abound, Gem, Headline, Pristine or Quadris Top) without alternating to another fungicide with a different mode of action, currently ferbam and copper. To be particularly careful to conserve fungicide efficacy, it is recommended to rotate modes of action with each application.
4. 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; Pristine 74 oz./acre/season; Quadris Top 61.5 fl. oz./acre/season).
5. 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).
6. Do not apply strobilurin-containing products in nurseries. Application of strobilurin in nurseries can result in selection of resistant strains which are then distributed on nursery stock to groves.

An example of an application program in the time of strobilurin resistance

Products	Quadris Top or Pristine	Copper	Opposite premix	Copper	Ferbam	Copper	Quadris Top or Pristine
FRAC code	11/3 or 11/7	M1	11/7 or 11/3	M1	M3	M1	11/3 or 11/7