UF UNIVERSITY of FLORIDA | IFAS EXTENSION

Berry/Vegetable Times

Special Alert

Perspective on resistance of *Botrytis cinerea* from strawberry to multiple fungicides in Florida

Achour Amiri, Post Doctoral Scientist and Natalia Peres, Associate Professor, Univ. of Florida/GCREC
Alicia Whidden, Extension Agent Vegetables & Small Fruits, Hillsborough County

Strawberry growers in the Dover and Plant City area experienced an epidemic of Botrytis the last 10 days of February 2012. The economic consequences have yet to be determined but the immediate question is why such a bad epidemic happened? It is difficult to give an immediate answer without further analysis of many of the factors that might have contributed to this situation. Among the factors that should be considered are the specific weather conditions favoring Botrytis development at the peak bloom, the widespread or emergence of resistance to many fungicides commonly used against Botrytis, and perhaps inappropriate fungicide selection.

<u>A quick review of Botrytis fruit rot development:</u>

The fungus survives and produces spores on dead leaves and then forms latent (dormant) infections on flowers and fruit. The disease is favored by prolonged periods of high humidity (more than 14 hours) and temperatures around 68°F. Under these conditions, if flowers are infected and appropriate fungicide sprays are not applied immediately, Botrytis symptoms are usually observed within 15 to 20 days after infection.

During February, the Strawberry Advisory System for monitoring the risk for Botrytis and anthracnose (http://agroclimate.org/tools/strawberry/) showed two periods when leaf wetness duration and temperatures were highly conducive for Botrytis development. The first occurred on February 6 and 7 and corresponded to full bloom in 'Radiance' and the beginning of the peak bloom for 'Festival'. The second event occurred on February 17 and 18 and corresponded to the full bloom in 'Festival'. Growers in the Balm area suffered less from this Botrytis outbreak. According to the Strawberry Advisory System, there was only a moderate risk of disease development on February 7 and conditions were highly conducive on February 17 which may explain the lower disease

(Continued on page 2)

Spring 2012 brings new concerns for Spotted Wing Drosophila in berries

Jim Price, Univ. of Florida/GCREC

We have found natural infestations of spotted wing drosophila (SWD) larvae in field grown strawberry fruit at GCREC and we continue to catch adult SWD flies in our traps for the first time during the winter strawberry crop. These are indications that the fly will be more problematic this season than in the colder prior years of the infestation.

Blueberry, raspberry, and blackberry growers should seek UF Extension advice, monitor for the fly, and respond with appropriate insecticides and other actions as conditions warrant. The recent UF IFAS publication http://edis.ifas.ufl.edu/in839 reveals the best practices available to avoid losses in berry crops.

A University of Florida/IFAS and Florida Cooperative Extension Service Newsletter
Hillsborough County, 5339 CR 579, Seffner, FL 33584
(813) 744-5519
Alicia Whidden, Editor
Gulf Coast Research & Education Center, 14625 County Road 672, Wimauma, FL 33598
(813) 634-0000
Jack Rechcigl, Center Director
Christine Cooley, Layout and Design
James F. Price, Co-Editor
http://gcrec.ifas.ufl.edu

incidence in the Balm area.

Botrytis fruit rot control:

Several studies have demonstrated that fungicides should be applied at the peak bloom to control Botrytis. Thus, recommendations for control are based on applications of protective fungicides such as captan and thiram early and late in season and application of more effective single-site-of-action fungicides during the main peak bloom period. Fungicides registered for control of Botrytis fruit rot in Florida are listed in Table 1.

Understanding fungicide resistance:

Fungicides that have a single-site mode of action are active at low concentrations but are usually considered as 'high-risk' for resistance development. Thus, repeated applications of these fungicides may select for mutations in the gene targeted by the fungicide. These mutations result in reduced efficacy which may ultimately result in a total control failure. Because of its abundant sporulation, high genetic variability, and wide host range, *Botrytis cinerea* is also considered as a 'high risk' pathogen for resistance development. In fact, resistance or reduced sensitivity of Botrytis to some of the fungicides mentioned in Table 1 has already been reported in other parts of the world on many crops including strawberries. However, little was known about the distribution of Botrytis-resistant populations in strawberry fields in Florida.

Screening for fungicide sensitivity of *B. cinerea* isolates from Florida

As part of a grant funded by the USDA-NIFA Specialty Crops Program, a research project was started to evaluate the potential for resistance development in *B. cinerea* from Florida. Two hundred and fifty-two (252) Botrytis isolates collected from different strawberry fields between 2001 and 2012 have been evaluated in a three-step method: (1) each isolate is tested for its sensitivity to each fungicide using a fungicide-agar assay; (2) a representative sub-sample of isolates having different levels of sensitivity to each fungicide is tested on detached strawberry fruits sprayed with the fungicide at the recommended field rate. This step helps in selecting a dose of fungicide that can discriminate a sensitive from a resistant isolate (Figure 1) when grown on agar medium and also verifies the ability or the failure of the fungicide to control all isolates; (3) the mutation in the target gene is verified using molecular tests. This three-step methodology is robust enough to gather solid evidence on actual resistance development.

Our preliminary results have shown different frequencies of resistance of *B. cinerea* isolates from Florida strawberry fields to all fungicides tested with the exception of Switch and fludioxonil (one of the components of Switch). The highest resistance frequencies were observed for Topsin M (100%), Pristine (83%) followed by Scala (60%). Overall, about 50% of the isolates were resistant to Elevate and cyprodinil (a component of Switch). The frequency of Botrytis isolates sensitive or resistant to six selected fungicides is shown in Figure 2.

Results for the occurrence of resistance to Pristine and its two different components have already been reported at the last Agritech meeting in August. The screening of new fungicides belonging to fungicide group 7 (same as the boscalid, a component of Pristine) that are coming onto the market in the near future were also presented. A reference was also made at the time about the ongoing screening with Scala, cyprodinil (a component of Switch), and Elevate. The two first fungicides are from the same chemical group (9) meaning that they have similar mechanisms of action.

Therefore, cross-resistance has certainly played a role in resistance development to cyprodinil and Scala. In addition, cyprodinil is also a component of Inspire Super, a fungicide just recently registered for strawberries and cross resistance should also be expected. The emergence of resistance to Elevate has just been confirmed in the last month by a molecular assay which revealed the presence of a mutation in the targeted gene. Our preliminary results revealed higher resistance frequencies of Elevate in strawberry fields were frequent applications of this fungicide were made. So far, none of the isolates tested so far were resistant to fludioxonil, the main active ingredient in Switch, when tested using the agar test and all isolates were also fully controlled either by fludioxonil alone or Switch when tested on fruits.

Implications of the recent outbreak and research findings:

The results described above are from ongoing research that indicate widespread resistance of *B. cinerea* to many fungicides used in Florida strawberry fields. The good news is that not all isolates are resistant to all fungicides. Our preliminary results have shown that an average of 15 to 20% of isolates were resistant to all fungicides except Switch. Consequently, some of these fungicides should give at least partial control of the disease. However, choosing this strategy will worsen resistance development as more resistant isolates will be selected. Moreover, when high disease pressure persists in the field for several days, these fungicides will not be effective against the remaining 15 to 20% of resistant isolates and this is enough to create a control failure.

Under environmental conditions favoring epidemics and considering the widespread resistance in *B. cinerea* to several fungicides, it becomes very critical to rethink our spray program recommendations. For this, knowledge of the resistance scenario for each strawberry field may become crucial for appropriate control recommendations. Research is currently underway to develop a quick and reliable field kit to determine location-specific resistance profiles for all fungicides. Our hope is that this tool will permit scientifically based recommendations for individual strawberry fields, i.e., a resistance profile will be generated for each fungicide for a particular field and consequent spray recommendations will be given based on the actual resistance scenario found.

In addition, to reduce risks of fungicide resistance development and prevent future epidemics, timing fungicide applications properly will be crucial. In another words, fungicides will need to be applied only when necessary. For this, the Strawberry Advisory System (SAS) has been developed to alert growers of conditions conducive for Botrytis development. The system is currently being tested in different commercial strawberry fields. In most cases, the epidemic of Botrytis was just as bad in the grower standard treatment that received weekly applications of fungicides as it was in the areas that received fungicide applications according to the SAS recommendations. However, fewer fungicide applications were generally used when following the SAS recommendation and, consequently, the risk for fungicide resistance development would also be reduced.

The population of *B. cinerea* evolves quickly and differs from year to year. In Florida, new inoculum is possibly introduced every year with new strawberry transplants. Thus, more research will be needed to evaluate whether the inoculum coming on the transplants is already resistant to certain fungicides or if the resistant population is remaining in Florida strawberry fields from one season to the next.

Results from our ongoing investigations on fungicide resistance will be presented in the upcoming Agritech meeting in August 2012.

Table and figures on Pages 4 and 5.

Table 1. Fungicides registered for Botrytis control in Florida

Trade name	Active ingredient	Fungicide group	Recommended Field rate /ac	Maximum number applications/season	Risk for resistance
Thiram Granuflo	thiram	M3	4.4 lb	5	Unknown
Captan, Captec	captan	M4	1.88 to 3.75 lb		Unknown
Pristine	boscalid + pyraclostrobin	7 + 11	23 oz	5	High
Scala SC	pyrimethanil	9	18 oz	3	Medium to high
Switch WG	cyprodinil + fludioxonil	9 + 12	14 oz	4	Low to medium
Inspire Super	cyprodinil + difenoconazole	9 + 3	20 fl oz	4	Medium
Elevate 50 WDG	fenhexamid	17	1.5 lb	4	Medium to high
Captevate 68 WDG	captan + fenhexamid	M4 + 17	5.25 lb	4	Low to medium
Topsin M WSB	thiophanate-methyl	1	1 lb	4	High

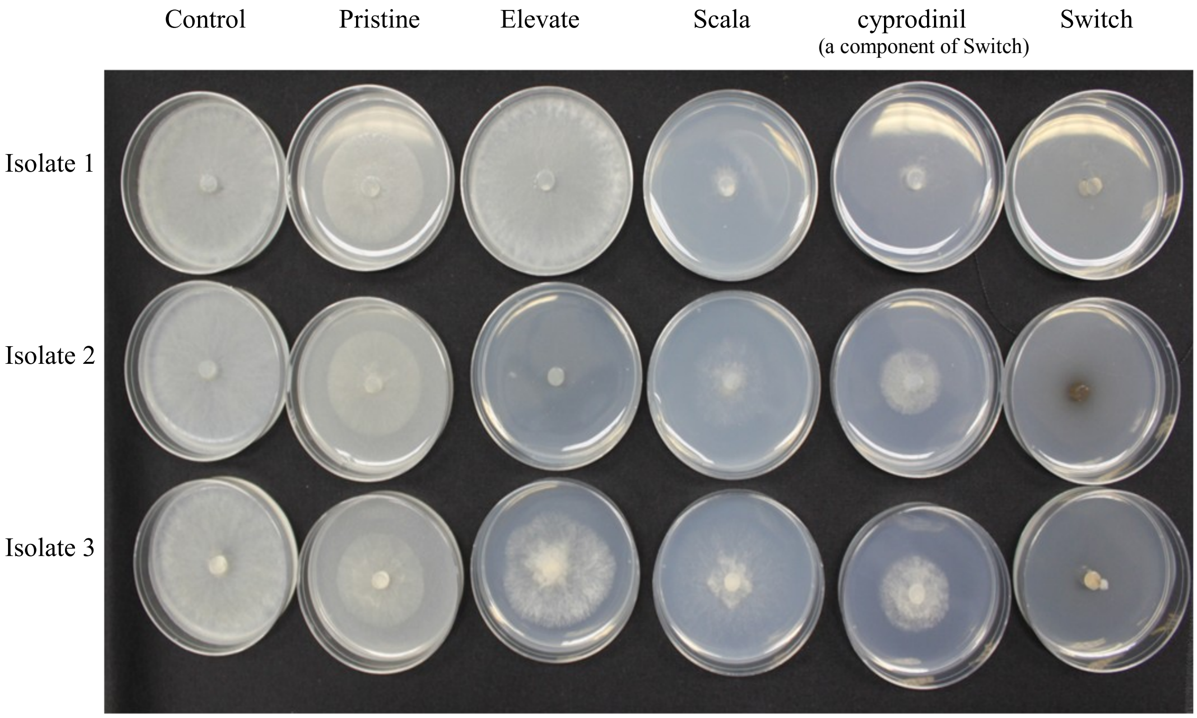

Figure 1. Discrimination of sensitive from resistant isolates of *B. cinerea* in an agar assay. The control plates do not contain any fungicide and are used to compare the growth on the plate amended with each fungicide. In this case, the 3 isolates are resistant to Pristine; isolate 1 and 3 are resistant to Elevate; isolates 2 and 3 are resistant to Scala and cyprodinil; all 3 isolates are sensitive to Switch.

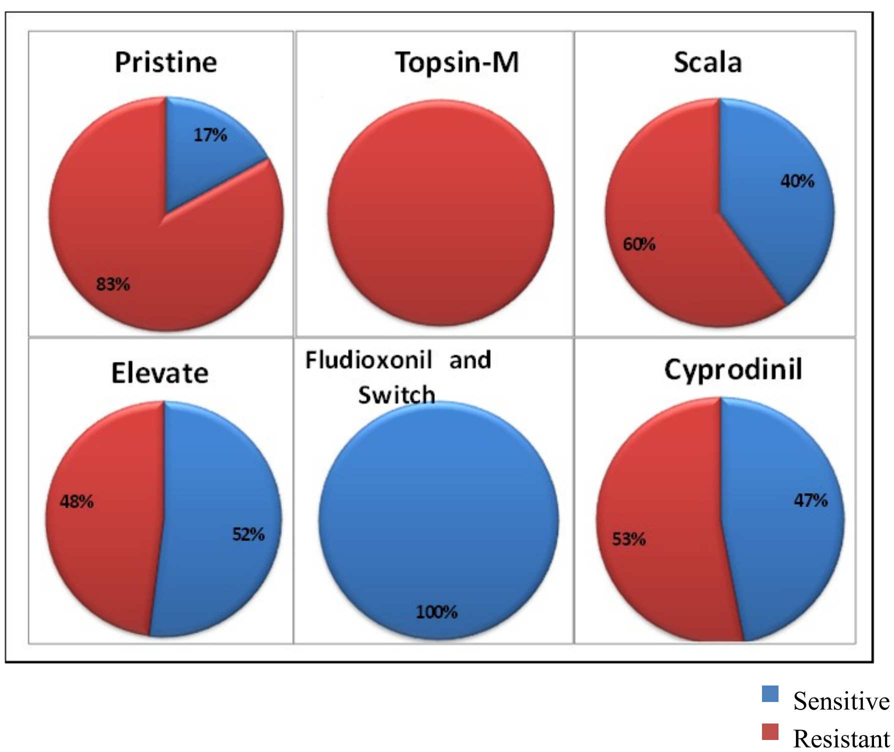

Figure 2. Frequency of sensitive and resistant isolates of *Botrytis cinerea* to different fungicides commonly used in strawberry fields in Florida. The study is based on 252 isolates including all strawberry fields surveyed. The results are from laboratory and fruit tests.