

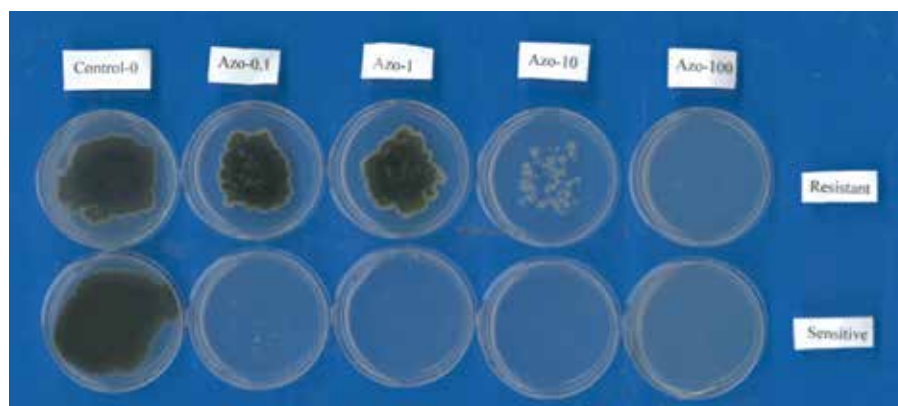
# PRINCIPLES OF FUNGICIDE RESISTANCE

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## FAST FACTS

- Fungicide resistant strains of a fungal plant pathogen are usually already in the population and caused by natural mutations — not caused by the application of fungicides.
- Fungicide applications select out these individual mutants, building up their population while reducing the population of the original sensitive wild-types.
- Once the population of fungicide resistant mutants is high, then the loss of fungicide efficacy occurs.

Fungicide resistance can be defined as when a pathogen population changes from being sensitive to a fungicide, to one that is insensitive or less sensitive to a fungicide. Resistance may not always be complete. When resistance is not complete in a fungus, that fungus may sometimes be referred to as having reduced sensitivity to a fungicide.



The graphic above shows an example of fungicide resistance in the fungus known as *Cercospora soja*, which causes frog-eye leaf spot of soybean. In this picture, an isolate resistant to the fungicide azoxystrobin is in the top row, while a sensitive isolate is in the bottom row. The concentration of azoxystrobin increases from left to right. As observed in this picture, the sensitive isolate is almost completely inhibited by 0.1 parts per million of azoxystrobin, while it takes 100 parts per million of azoxystrobin to almost completely inhibit the resistant isolate.

## FACTORS THAT AFFECT FUNGICIDE RESISTANCE

Fungicide resistance is affected by two types of factors: factors associated with the pathogen and factors associated with the fungicide.



### PATHOGEN FACTORS

An example of a factor associated with the fungal pathogen (that can influence the risk to fungicide resistance) includes a level of genetic diversity with that pathogen population. Fungal pathogens that go through sexual recombination may have a higher level of genetic diversity within that population. In general, fungicide resistance is more likely to occur within fungal populations that have a high level of genetic diversity compared to populations with low genetic diversity.

Fungicide resistance is also more likely to occur in fungi that have a short generation time. A shorter generation time means more that more individual isolates of the fungus will occur within a growing season. Thus allowing for more genetic diversity and a greater chance of mutation that could result into reduced sensitivity to a fungicide.

Fungi that produce a large number of spores also are more likely to develop fungicide resistance. Fungi that have several of these factors mentioned are those that would be considered to be at the highest risk for fungicide resistance to occur.

### FUNGICIDE FACTORS

An example of a factor associated with the fungicide that can influence the risk of fungicide resistance includes the fungicide site of action. Fungicides with a single site of action are at a higher risk of selecting out strains of a fungus that are resistant to the fungicide compared to fungicides with multiple sites of action.

Factors associated with a specific fungicide application can influence the risk of fungicide resistance. Fungicides that are applied more than once during a single season are at a higher risk for fungi developing resistance to them. If that fungicide had been used frequently within an area year after year, the risk becomes even greater.

Fungicides that typically are applied after disease symptoms are present—which can be considered a curative type of application—may be more prone to fungicide resistance. Allowing a disease epidemic to be in progress before making a fungicide application may allow the fungus to go through multiple cycles and be at a high population at the time when the fungicide application is made. In turn, this increases the opportunities for the appearance of a fungicide resistant mutant. Fungicides that are not applied in combination with different fungicide active ingredients from different fungicide groups are at higher risk for fungicide resistance occurring.

### FOLIAR FUNGICIDES APPLIED TO SOYBEAN + CORN IN THE U.S.

Currently, there are two primary chemical classes that are commonly used on soybean and corn as foliar fungicides in the United States. One of these major chemical classes is known as the quinone outside inhibitors (QoI). This group includes strobilurin fungicides. The other major chemical class is known as the demethylation inhibitors (DMI). This group includes the triazole fungicides.

Succinate dehydrogenase inhibitors (SDHI) is another class of fungicides that are becoming more important as active ingredients in fungicide products used on field crops. Other fungicides classes that are sometimes being used on corn and soybean as foliar fungicides include the methyl benzimidazole carbamates (MBC) and fungicides with multi-site contact activity, such as the chloronitriles.

### FUNGICIDE RESISTANCE ACTION COMMITTEE (FRAC)

To help sort out the different fungicide groups and develop fungicide resistance management guidelines, an organization known as the Fungicide Resistance Action Committee (FRAC) was developed. FRAC developed a code that uses numbers and letters that helps to distinguish the different fungicide groups. This code is known as the FRAC code. Fungi resistant to a fungicide within a specific FRAC code may be resistant to all fungicides within that FRAC code. This phenomena often is referred to as cross-resistance.



The FRAC code, generally, will appear on the front of the fungicide label. Here are some examples of labels of different fungicide products that show the FRAC code. For example, the fungicide shown as exhibit A (as seen in the upper left hand corner) contains a single active ingredient that is in FRAC group 11. To the right of that is B, which contains a single active ingredient that is in FRAC group 3. The bottom examples show fungicide products that contain

two different active ingredients from different groups. On the bottom left hand corner is C, which contains fungicides from FRAC groups 3 and 11. On the bottom right hand corner is D, which contains fungicides from FRAC groups 7 and 11.

FRAC Code	Fungicide Group	Risk of Resistance Developing
1	Methyl benzimidazole carbamates (MBC)	High
3	Demethylation inhibitors (DMI, includes triazoles)	Medium
7	Succinate dehydrogenase inhibitors (SDHI)	Medium to High
11	Quinone outside inhibitors (QoI, includes strobilurins)	High
M5	Chloronitriles	Low

FRAC has determined the risk level of fungicide resistance developing to each of the fungicide groups. This table shows the most commonly used fungicide groups that may be applied as a foliar fungicide to corner soybean in the United States. FRAC code 1, known as methyl benzimidazole carbamates (MBC), has a high risk of fungicide resistance developing. FRAC code 3, known as demethylation inhibitors (DMI—includes triazoles), has a medium risk. FRAC code 7, known as succinate dehydrogenase inhibitors (SDHI), has a medium to high risk. FRAC code 11, known as quinone outside inhibitors (QoI—includes strobilurins), has a high risk. And FRAC code M5, known as chloronitriles, has a low risk.

## HOW DOES FUNGICIDE RESISTANCE DEVELOP?

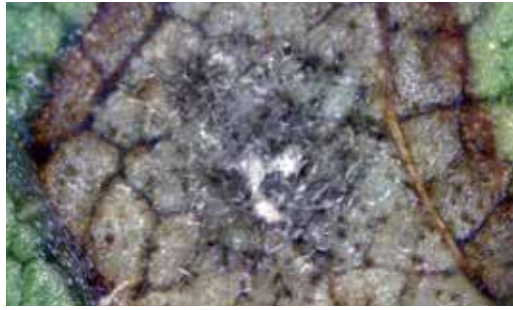
For fungicide resistance to develop, fungicide-resistant isolates must already be in the pathogen population. In general, natural mutations are responsible for fungicide-resistant isolates. It is important to note, the fungicides applied do not cause these mutations. These mutations occur naturally. In general, natural mutations occur in fungi at the approximate rate of 1/100,000,000.

To get a better idea of how often mutations may occur within a pathogen population, let's look at *Cercospora sojina*, which is a fungus that causes frogeye leaf spot on soybean. On a susceptible soybean variety, under favorable conditions, frogeye leaf spot may be severe. In this case, approximately 100 lesions or spots may be present on a soybean leaflet. Under the right conditions, each of these lesions may be sporulating. Sporulating means spores of the fungus are present on these lesions. These spores are also known as conidia.



If one were to look at these lesions with the aid of magnification, approximately 100 conidia per frogeye leaf spot lesion may be present. These conidia can be observed in the picture on this slide as a fuzzy mass seen in the middle of the lesion. This picture was taken through a dissecting microscope.

When conditions are favorable for frogeye leaf spot, approximately 30 leaflets per soybean plant could be affected. Considering that there may be approximately 6 million soybean plants in a 40-acre field, that is a lot of leaflets with sporulating



lesions. In fact, using these numbers, there would be approximately 1.8 trillion *Cercospora sojina* conidia coming from a 40-acre field. Considering that mutations occur at the approximate rate of one out of 100 million, that would mean that approximately 18,000 mutant isolates may occur within a 40-acre field. Some of these naturally occurring mutants may be less sensitive to fungicides.

## HOW DOES FUNGICIDE RESISTANCE DEVELOP?

If natural mutation results into a few isolates that are resistant to a fungicide, foliar fungicides may still be effective for a while (as long as there are only a few fungicide resistant isolates in the field). However, when a fungicide is applied a selection pressure is applied to that fungal population in the field. If mutant fungicide resistant isolates are already in the field, then a fungicide application will select out those individual isolates that are resistant to the fungicide while killing of some of the sensitive isolates.



This graphic shows an example of how selection pressure may occur. As seen in the first soybean leaflet, conidia or spores are present. These conidia are shown in two different colors. The green conidia represents those that are still sensitive to a fungicide, and the red conidia represent those that are resistant to fungicide.

A fungicide is then applied to the field. This fungicide applies a selection pressure. As seen in the second soybean leaflet, there are now fewer green or sensitive conidia, but the red or fungicide resistant conidia remains. These remaining conidia re-infect leaves and cause symptoms. The fungicide is then again applied to the field. This time more of the red or fungicide resistant conidia are selected out of the population, while many of the green or sensitive conidia were killed. Continued use of the selection pressure, also known as the fungicide, will continue to select out the fungicide resistant individuals in the fungal population. Eventually, the resistant isolates will dominate the population and the effectiveness of the fungicide will be reduced.

As mentioned previously, the risk of resistance to QoI fungicides is considered high. Resistance to QoI fungicides are due to single-step amino acid substitutions that occur in the fungal cytochrome b gene. Three different mutations in the cytochrome b gene have been documented.

The mutation that has been observed most often in fungi that have resistance to QoI fungicides is known as the G143A mutation. Fungi with this mutation may have complete resistance to QoI fungicides. The G143A mutation occurs when there is a change from the amino acid glycine to alanine at codon 143 in the cytochrome b gene of the fungus.

The F129L mutation is another type of mutation that may cause a fungus to be resistant to QoI fungicides. This mutation has been observed less often, and fungi with this mutation are more likely to have partial resistance to the fungicide rather than complete resistance. This mutation occurs when phenylalanine is substituted with leucine at codon 129.

The G137R mutation has been observed rarely in fungi. Fungi with this mutation may have partial resistance to QoI fungicides rather than complete resistance. This mutation occurs when the amino acid glycine changes to arginine at codon 137.

The risk of resistance to DMI fungicide is considered medium. Resistance to DMI fungicides are due to several mechanisms often associated with affecting the sterol 14 alpha-demethylase (CYP51) enzyme.

This enzyme is involved in the production of ergosterol in fungi.

Resistance to DMIs is a multi-step process, and is observed as a slow erosion of fungicide efficacy over time. This means that the fungal population becomes less and less sensitive to the DMI fungicide over time.

Resistance to SDHI fungicides is complicated and not as well understood as resistance to QoI fungicides. Strains of fungi with reduced sensitivity to a specific SDHI fungicide active ingredient may not necessarily have reduced sensitivity to all SDHI fungicide active ingredients.

## FUNGICIDE RESISTANCE CONCERNS IN SOYBEAN + CORN PRODUCTION

In general, fungicide resistance in soybean and corn production has not been considered as important historically. Especially in comparison to more intensively managed crops and plants. This perception has existed because most corn and soybean acres in the United States are not treated with a foliar fungicide. In addition, when corn and soybean fields are treated with a foliar fungicide, generally only one application per season is made.

Regardless of perception, fungicide resistance can occur in pathogens of soybean and corn. The widespread occurrence of QoI fungicide resistance *Cercospora sojina* confirms this can happen.

## FUNGICIDE RESISTANCE MANAGEMENT

Fungicide resistance can be difficult to prevent, but using certain management practices to help retain the efficacy of fungicides for a long period of time is possible. These management practices include the following:

- » Utilize non-chemical methods of disease management (this includes planting resistant cultivars and crop rotation)
- » Apply products that contain mixtures of different fungicide classes or rotate fungicide classes if more than one application is used in a season
- » Follow label recommendations
- » Use integrated pest management (IPM) practices and only apply a fungicide when warranted based on disease scouting observations and disease risk

### ADDITIONAL RESOURCES

Fungicide Resistance Action Committee Website. [www.frac.info](http://www.frac.info).

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Mueller, D. S., Wise, K. A., Dufault, N. S., Bradley, C. A., and Chilvers, M. I. 2013. Fungicides for Field Crops. American Phytopathological Society, St. Paul, MN.

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Zhang, G., Pedersen, D. K., Phillips, D. V., and Bradley, C. A. 2012. Sensitivity of *Cercospora sojina* isolates to quinone outside inhibitor fungicides. Crop Prot. 40:63-68.

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