

PECAN SCAB: UNDERSTANDING FUNGICIDE ACTIVITY TO PREVENT FUNGICIDE RESISTANCE

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Introduction: Fungicides labeled for control of pecan scab differ in their chemical properties, their activity on the fungus, and the ease by which the pathogen can become resistant to them. It is important to understand this relationship so that the best fungicide choices can be made for control of scab. Some fungicides with excellent control properties against scab are also highly prone to resistance. There are three categories of fungicide resistance risk to pecan scab, which are shown in Table 1. To better understand why risk of resistance differs among fungicide groups, the mechanisms of fungicide action and resistance mechanisms of the fungus will be discussed in detail for each pecan fungicide. Relationships are summarized in Table 2.

Recommendations for preventing fungicide resistance:

- * Apply moderate or high risk fungicides in alternation with fungicides that have a different mode of action. E.g. Abound followed by Orbit, but NOT Abound followed by Sovran (different fungicides, same mode of action).
- * Use mixtures of fungicides with different modes of action. E.g. Stratego (= propiconazole + trifloxystrobin), Quilt (= azoxystrobin + propiconazole), Enable/Agri-Tin package.
- * Limit the number of moderate and high risk fungicides used in a season.
- * Do not use fungicides to cure infections, apply before symptom appearance.
- * Do not use a lower rate than recommended by the label.
- * Use spray advisories or disease forecasting systems to minimize the number of fungicide applications.

Table 1. Relative risk of fungicides for resistance development by the pecan scab fungus, *Fusicladosporium effusum* (synonym: *Cladosporium caryigenum*).

Risk Category	Site of Action	Resistance Response by the Fungus	Fungicide Classes (trade name)
Low	Many	Too many genes involved: resistance is unlikely and the population is always sensitive	Dithiocarbamate (Ziram); Organometallic (Agri-Tin, Super-Tin)
Moderate	A few or one	Quantitative: Several genes confer a gradual resistance development and it may not last within the population	Triazole (Enable, Orbit, Propimax, Folicur, Tebuzole, Orius, Monsoon, Toledo); Guanidine (Elast)
High	One	Qualitative: One gene involved, develops quickly and is usually permanent within the population	Benzimidazole (Topsin-M); Strobilurin (Abound, Sovran, Headline)

CONCEPTS OF FUNGICIDE RESISTANCE

Mutants - The basis for fungicide resistance: Some fungicides have activity against many different components of the fungus (i.e. many sites of action) and it is highly unlikely that a fungus will have all the mutations necessary to overcome this, so resistance is unlikely. Fungicides with one or a few sites of action in the fungus are prone to resistance because there is a high likelihood that there will be a few mutant individuals in the fungal population. After fungicide treatment, these individuals will survive and will likely reproduce.

Types of resistance: There are two types of resistance, qualitative and quantitative. With quantitative resistance, several genes each contribute a small degree of resistance. If individuals with several of these genes escape full control with the fungicide, they will have a competitive advantage. Sensitive individuals will die out and the population of survivors will have high numbers of resistant individuals and they will eventually dominate the population. Some species of fungi with resistant genes can still be controlled by the fungicide, except higher doses will be required. Eventually, the dose required exceeds what is economical or what is labeled. With qualitative resistance, a mutation in a single gene gives a high level of resistance and no increase in dose will affect the fungus. Such surviving mutants will quickly become dominant in the population and subsequent attempts at control will fail.

If quantitative resistance develops in a population, alternation with fungicides having a different mode of action will result in good control, but this strategy will not work if there is qualitative resistance in the population. With quantitative resistance, in addition to using high doses, the fungicide needs to be applied before infection has occurred, otherwise sensitive isolates could develop lesions and reproduce, giving the opportunity for resistance selection.

Resistance penalty: Sometimes the appearance of fungicide resistance is also associated with other traits, notably reduced pathogenicity and fitness in the environment when the fungicide is no longer used. This is known as a “resistance penalty”. It has been reported for fungicides generating quantitative resistance in fungi, such as the triazole fungicides (e.g. propiconazole). Knowing this can be used to an advantage. For example, a shift towards resistance could be managed with higher doses of the fungicide. Additionally, fungicides no longer used because of resistance could be re-introduced in the future because the resistant mutants, in the absence of the factor that favors their growth, become a small part of the pathogen population. However, there is also the possibility that additional mutations could overcome reduced fitness in the environment.

Cross resistance: In this article, I have emphasized the chemical class of the fungicides. There are different fungicides that fall into the same chemical class. It is extremely important to be aware this because resistance of a fungus to one chemical in a class means that the fungus is essentially always resistant to all other fungicides in that class. This is known as “cross-resistance”. In terms of fungicide resistance management for disease control, it is not helpful to use a different fungicide if it is in the same class; it is essential that the other fungicide(s) are in different chemical classes, with different modes of action.

FUNGICIDES LABELED FOR CONTROL OF PECAN SCAB: THEIR MECHANISM OF ACTION AND MECHANISM OF FUNGUS RESISTANCE

Table 2. Summary of relative risk of fungicides for resistance development by the pecan scab fungus (*Fusicladosporium effusum*, synonym *Cladosporium caryigenum*), mode of fungicide action, and mechanism of pathogen resistance.

Fungicides*	Resistance Risk	Mode of Fungicide Action	Mechanism of Pathogen Resistance
Topsin-M	High	Interferes with cell division by binding to β -tubulin protein.	Change of β -tubulin structure prevents binding.
Abound, Sovran, Headline	High	Interferes with energy production by binding to cytochrome b (Q_o site) in the mitochondrion.	Change of cytochrome b structure prevents binding.
Enable, Orbit, Orius Bumper, Propimax, Folicur, Tebuzole, Monsoon, Toledo	Moderate	Prevents synthesis of ergosterol, a component of fungal membranes, in several ways.	Several: binding prevention, alternative synthesis pathways, fungicide removal from cell.
Elast	Moderate	Interferes with function of membranes and enzymes.	Not known.
Ziram	Low	Binds to proteins (enzymes), interfering with their function.	No field resistance known.
Agri-Tin, Super-Tin	Low	Interferes with energy production in the mitochondrion, in many ways.	Field resistance only in one species of pathogen.

***Stratego** and **Quilt** are mixtures. There is a low risk of resistance with these fungicide mixtures.

High-Risk Category for Resistance:

Topsin M (chemical name: thiophanate-methyl) - *Benzimidazole* chemical group

How it affects fungi: Inhibits spore germination and growth.

Mechanism of action: This fungicide interferes with cell division. It binds to certain portions of a protein involved in cell division, β -tubulin. This binding prevents the assembly of this tubulin protein with another tubulin protein, α -tubulin. These two proteins normally assemble into microtubules, which are involved in spindle formation. The spindles coordinate chromosome separation during cell division. Cells can not divide. This also interferes with

DNA synthesis, which is necessary for production of proteins, including enzymes. Enzymes are essential to regulate cell metabolism.

Animal and plant cells also have tubulin proteins. However, these tubulins differ in chemical structure from that of fungal tubulins. Certain amino acids at critical binding sites of the tubulin are required for the fungicide to bind; they are present in fungi and lacking in other organisms.

Type of resistance: Qualitative.

Mechanism of resistance: A single mutation in the DNA of the fungus that results in changes in two amino acid positions (#198 and #200) of the β -tubulin gene. This changes the structure of the tubulin. The fungicide can no longer bind to it.

Cross-resistance is well documented in this class of fungicides. For example, if a fungus is resistant to Benlate (benomyl), it will most likely be resistant to Topsin M (thiophanate-methyl).

Status of this fungicide for pecans: Benlate (benomyl) was first introduced for pecan scab control in the early 1970's. Resistance was identified in pecan scab isolates from Georgia in 1975, from orchards with a consistent and heavy use of benomyl, particularly when it was the sole fungicide used. Benlate is no longer available, but in orchards where it was used extensively, it is likely that strains of the fungus resistant to Topsin M will be prevalent, or will become prevalent after only one fungicide application.

Abound (chemical name: azoxystrobin) - *Strobilurin* chemical group

Sovran (chemical name: kresoxim-methyl) “ ” “

Headline (chemical name: pyraclostrobin) “ ” “

Stratego (chemical name: trifloxystrobin) “ ” “

Quilt (chemical name: azoxystrobin) “ “ “

(NOTE: Stratego and Quilt are mixtures that also contain propiconazole)

How it affects fungi: Inhibits spore germination and growth.

Mechanism of action: This fungicide interferes with energy production (mitochondrial respiration). It binds to a specific part of the mitochondrion, the Q_o site (the outer, quinone oxidizing pocket) of cytochrome b, which is a part of the cytochrome bc_1 complex, located in the inner mitochondrial membrane of fungi and other eukaryotes (plants and animals). When the fungicide binds, it blocks electron transfer between cytochrome b and cytochrome c_1 . This stops the production of ATP, which disrupts energy production. This shuts down everything else.

Type of resistance: Primarily qualitative, i.e. a change in one gene. A quantitative form of resistance has also been described for the apple scab fungus, *Venturia inaequalis*.

Mechanism of resistance: The major mechanism, which has been reported for several species of fungi, is a single mutation in the mitochondrial b gene. This change in the gene results in an amino acid substitution from glycine to alanine at position 143 of the cytochrome b protein. This mutation is referred to as “G143A”. The fungicide can no longer bind to the Q_o site.

Yet another reported mutation results in the substitution of phenylalanine with leucine at position 129 of cytochrome b (mutation “F129L”). Again, the fungicide can not bind to the Q_o site.

When azoxystrobin, the first commercial strobilurin fungicide, was introduced into the marketplace, the researchers behind its development thought that resistance would be an incremental process. A mutation for resistance could occur in the DNA of one mitochondrion, but many other mitochondria in a cell would still be sensitive. Furthermore, the mutation for resistance would be associated with reduced fitness in one or more other traits, which would make the mutant less competitive. This would slow the development of field resistance.

In fact, resistance to strobilurin fungicides in many different fungal pathogens has appeared much faster than anticipated and the appearance of resistance has been more sudden than gradual. This could be demonstrated within one growing season. For example, the frequency of G143A mutant genes in field populations of the wheat powdery mildew fungus, *Blumeria graminis* f.sp. *tritici*, increased from 2.2% to 58% following the selection pressure from three successive applications of a strobilurin.

Because strobilurin resistance is such a new phenomenon, there is no long-term information about whether there is a resistance penalty associated with these fungicides. With the wheat powdery mildew fungus, there appears to be a fitness penalty to strobilurin resistance. In one study, a reversion to the wild type was observed in strobilurin-resistant populations after 30 generations without exposure to strobilurins. This reversion occurred through mutations back to the original form and subsequent selection by the environment.

Although the three strobilurin fungicides labeled for pecans function as Q₀ inhibitors, they have different chemical properties and levels of action against fungi. However, because of their common mode of action, they exhibit cross resistance.

Status of this fungicide for pecans: No resistance to strobilurins documented at this time.

Moderate-Risk Category for Resistance:

Orbit, Propimax, Bumper (chemical name: propiconazole) - *Triazole* chemical group

Folicur, Tebuzole, Monsoon, Orius, Toledo (chemical name: tebuconazole) “ “

Enable (chemical name: fenbuconazole) “ ” “

Stratego, Quilt (chemical name: propiconazole) “ ” “

(NOTE: *Stratego* also contains trifloxystrobin and *Quilt* contains azoxystrobin)

How it affects fungi: Inhibits growth, but not spore germination.

Mechanism of action: This fungicide prevents the fungus from synthesizing ergosterol, an important component of cell and mitochondrial membranes, by inhibiting the reaction catalyzed by the enzyme, 14- α -demethylase. Specifically, it inhibits the cytochrome P450 dependent oxidative demethylation of eburicol, because the heterocyclic ring of the fungicide binds to the sixth ligand position of the P450 heme iron. This binding simultaneously prevents substrate binding and oxygen activation. When the ergosterol biosynthesis pathway is blocked at this point, the fungus accumulates 14-methyl-3,6-diol (through the reaction of the enzyme C5-6 desaturase) as well as lanosterol, and both sterols have detrimental effects upon membranes.

Since spores have their own reserve of ergosterol to draw upon, germination is not affected. As growth requires new ergosterol synthesis, germinated spores can not grow further.

Fungicides in this chemical group, as well as those in other chemical groups, that have this mechanism of action are known as “demethylation inhibitors”, or “DMI” fungicides, or “sterol inhibitors”, or “SI” fungicides.

Type of resistance: Quantitative; resistant populations respond to high doses of the fungicide.

Mechanism of resistance: Several mechanisms have been described, including mutations in different genes encoding enzymes for ergosterol biosynthesis. For example, a mutation in codon 136 of the gene that encodes for cytochrome P450 monooxygenase has been reported. Another mutation affecting cytochrome P-450 14 α -demethylase changes its conformation (shape) so that the fungicide doesn't bind to it, but this enzyme also becomes incapable of catalyzing C-14 demethylation and this reduces the vitality of mutants.

Another mutation allows the fungus to circumvent the pathway affected by the fungicide. This single-gene mutation at sterol C5-6 desaturation of the ergosterol biosynthesis pathway causes fungicide-treated mutants to accumulate 14-methyl fecosterol. This is a functional sterol for growth and can substitute the depleted ergosterol or stabilize the membrane to protect it from the disruptive effects of sterol precursors. Demethylation is still inhibited, but the C5-6 desaturase enzyme is also blocked, so toxic sterols do not accumulate.

There are other mechanisms that do not involve changes in the pathway of ergosterol synthesis. This includes an active efflux (removal) mechanism. The removal of the fungicide requires energy and may decrease fitness of the fungus in the environment. Alternatively, there may be structural or biochemical modifications of the cell wall that prevent penetration of the fungicide. Or, the fungus may be able to detoxify the fungicide.

Cross-resistance has been reported for this group of fungicides.

Status of this fungicide for pecans: A survey of Georgia orchards in 2002 found unsatisfactory control of scab in some orchards with triazole fungicides. In 6 of 7 orchards with a history of triazole use, a decline of sensitivity of the pathogen occurred from 1995 to 2003. There was no decline in sensitivity of the pathogen sampled from orchards with no use of triazole fungicides.

Elast - (chemical name: dodine) - *Guanidine* chemical group

How it affects fungi: Inhibits spore germination, prevents sporulation.

Mechanism of action: Multi-site. The basic guanidino group of the molecule binds to anionic groups, RCOO^- and ROPO_3^- , associated with membranes in the cell. This alters the membrane, allowing leakage of low molecular weight metabolites and potassium, and allowing entrance of sugar phosphates and other compounds that don't normally cross the membrane. When it alters the plasma membrane, more fungicide is able to move into the cell. Enzymes are also inhibited, for example, enzymes affecting glucose oxidation and RNA synthesis.

Type of resistance: Quantitative.

Mechanism of resistance: Mutations in four different genes have been reported, they work additively. The details of how these mutations confer resistance has not been reported.

Cross-resistance to guazantine has been reported.

This fungicide is exceptional in that it is a multi-site fungicide and, yet, pathogen resistance has developed in the field.

Status of this fungicide for pecans: No resistance of the scab pathogen has been reported.

Low-Risk Category for Resistance:

Ziram - (chemical name: zinc dimethyldithiocarbamate) - *Dialkyldithiocarbamate* chemical group

How it affects fungi: Inhibits spore germination and growth.

Mechanism of action: Multi-site. The mechanism of action is not well understood. It reacts with -SH groups of proteins, as well as metals attached to proteins. This would interfere with enzyme function. For example, it inhibits aconitase, an iron-dependent enzyme, probably by exchanging the zinc for the ferric ion of aconitase (i.e. removing the iron co-factor). This interferes with the tricarboxylic acid cycle. It may also form free radicals that react with cellular components.

Type of resistance: No field resistance has been documented.

Agri-Tin, Super-Tin - (chemical name: triphenyltin-hydroxide) - *Organometallic* chemical group

How it affects fungi: Inhibits spore germination and kills germinating spores.

Mechanism of action: Multi-site. The mechanism of action is not fully understood, but this fungicide ultimately interferes with energy production. The target site in the cell is the mitochondrion, including its membrane. It inhibits oxidative phosphorylation. It also promotes exchange of hydroxide anion across the lipid membranes, which uncouples enzyme reactions and alters substrate concentration inside the mitochondrion. It also causes swelling of mitochondria, leading to structural damage.

Mechanism of resistance: Field resistance has only been reported in the sugarbeet pathogen, *Cercospora beticola*. The resistant isolates are less competitive than sensitive isolates and do not persist in the environment once triphenyltin-hydroxide is no longer used. The resistant strains are cross-resistant to oligomycin, a non-related chemical that uncouples oxidative phosphorylation. This could mean that resistant strains would have resistance to any fungicide that works by uncoupling oxidative phosphorylation.

Status of this fungicide for pecans: No resistance of the scab pathogen has been reported.

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