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Department of
Agriculture

Agricultural
Research
Service

1998–04

February 1999

Pecan Industry: Current Situation and Future Challenges, Third National Pecan Workshop Proceedings

FUNGICIDE RESISTANCE MANAGEMENT IN PECANS

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Additional index words. Pecan scab, disease control, fungicide sensitivity

Introduction

Production of high quality pecans, particularly in the southeastern U.S., is dependent on availability of effective fungicides for disease control. A potential threat to the effectiveness of fungicides is the development of resistance to these compounds in pathogen populations. Fungicide resistance builds up in a pathogen population through the process of selection during repeated exposure to fungicide treatment, leading to the survival and spread of initially rare mutants (Brent 1995). Field resistance occurs when the frequency of resistant individuals in the pathogen population reaches a high level, resulting in substantially reduced efficacy or complete failure of the fungicide. The development of resistance may arise rapidly through qualitative population change (due to mutations in a single gene), or more gradually through a quantitative population change (controlled by multiple genes) (Köller 1991). The speed at which resistance develops is also influenced by epidemiology of the disease, susceptibility of the host, characteristics of the fungicide and the mode of application.

As a direct result of hard lessons learned from benomyl and other early systemic fungicides during the 1960's and 1970's, there is a heightened awareness in the agricultural community of the potential for resistance and the economic consequence of its occurrence (Russell 1995). The data collected over the past twenty five years have provided convincing evidence in support of the need for development and application of appropriate resistance management programs to prolong the effective life of new and existing agricultural fungicides.

Assessing the Risk of Fungicide Resistance

Most manufacturers of agricultural fungicides now include assessment of resistance risk and resistance management as a routine part of the development, labeling and marketing of new fungicides, well before the product is available on the market. Although it is difficult to predict reliably, the relative risk of resistance to new fungicides can be assessed based on characteristics of the target fungal pathogen, properties of the fungicide, and other factors associated with fungicide application (Brent 1995).

Pathogen and disease-related factors. The most important characteristics of the pathogen and disease that influence the likelihood of resistance are the number of reproductive cycles completed by the pathogen during a season and the potential for genetic variation in the pathogen population. High reproductive capacity leads to rapid population growth and provides increased opportunity for exposure of large populations to the selective pressure of fungicide exposure and rapid multiplication of resistant mutants. Populations of fungi that reproduce sexually are likely to be more genetically variable than those that reproduce only asexually. Greater genetic variation within a pathogen population provides greater opportunity for emergence of resistant mutants.

Fungicide and applications-related factors. The fungicide-related factors that are most closely associated with the risk of fungicide resistance development are the chemical class and mode of action of the compound and the frequency of application. A greater risk of resistance is associated with fungicides that have a very specific biochemical target site of action compared to those that have a non-specific mode of action. In general, resistance will develop much faster in pathogen populations that are exposed to more frequent applications of fungicide than in those that are exposed infrequently.

General Strategies for Resistance Management

Fungicide resistance management strategies are aimed at delaying, not preventing, the development of resistance. The only way to prevent the occurrence of fungicide resistance is to not use the fungicide. However, the careful use of at-risk fungicides can reduce the risk of resistance and delay the occurrence of resistance problems in the field. Resistance management strategies are most effective when applied prior to any detection of resistance. Once resistance has developed in a pathogen population, it is extremely difficult or impossible to eliminate it. Therefore, it is very important that resistance management strategies be implemented prior to detection of resistance (Bertrand and Padgett 1997).

General guidelines for fungicide resistance management can be summarized as follows (Brent

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1995):

1. *Do not use an at-risk fungicide in isolation.* Exclusive use of an at-risk compound places great selection pressure on the pathogen population and should be avoided. At-risk compounds should always be applied in combination with an appropriate fungicide partner. An appropriate partner is one that is chemically unrelated to the at-risk fungicide (i.e., a different mode of action), is not cross-resistant with the at-risk fungicide, and preferably has a non-specific mode of action with a relatively low risk of resistance. The choice of a appropriate partner with systemic, curative or protectant activity will also depend on the use situation (Urech 1988). Fungicide partners can be applied either as a tank mix (usually at reduced rates of both compounds) or applied in alternation, either as single applications, or in blocks of two or more applications of each fungicide.

2. *Restrict the number of applications per season.* Reducing the number of applications of an at-risk fungicide will help to reduce exposure of the pathogen population to selection pressure imposed by the fungicide.

3. *Maintain recommended dose.* Use of less than effective doses will lead to larger pathogen populations and increase exposure to the fungicide. In addition, use of less than effective doses may encourage the development of resistance through quantitative population changes. On the other hand, very high doses place excessive selection pressure on the pathogen population and encourage rapid selection of very resistant individuals. Resistance that develops through qualitative population changes is especially favored by use of very high doses.

4. *Avoid eradicant use.* Application of fungicides with eradicant or curative activity made after infection has occurred and disease symptoms are visible result in exposure of larger pathogen populations to the selective pressure of the fungicide than when fungicide is applied as a protectant before infection occurs. Post-infection application should also be avoided when using reduced rate mixtures that include a partner that has no significant eradicant activity.

5. *Maintain chemical diversity.* Incorporation of several fungicides with different modes of action into a disease management program will help to delay development of resistance.

6. *Practice integrated disease management.* To prolong the useful life of any fungicide, it should only be applied when necessary, and always in combination with cultural practices, use of resistant cultivars, and other non-chemical means of disease control (De Waard et al. 1993). The development of successful disease forecasting systems may also help to optimize the use of fungicides.

Fungal Diseases of Pecan and Relative Risk of Resistance

The pecan is susceptible to attack by variety of different fungi. However, by far, the major target of fungicide use on pecan is the fungus *Cladosporium caryigenum*, the fungus responsible for pecan scab. Based on a survey of pecan growers in Georgia (Bertrand and Hadden 1992), seven fungicide applications are made, on average, to control scab during a single growing season, but as many as eleven applications may be made during an unusually wet season or on a highly susceptible cultivar. Multiple applications of fungicide are necessary because the fungus can complete many reproductive cycles during the course of a growing season (polycyclic) and young, susceptible pecan tissue (leaves or fruit) is produced throughout the season. Pathogen development and infection are favored by warm wet weather, and under optimal conditions the fungus can complete a reproductive cycle in as little as 7-10 days. Although sexual reproduction is not known to occur in this fungus, the capacity for asexual reproduction during a given growing season is quite high. This is especially true during wet years or on highly susceptible cultivars.

Pecan Fungicides and Relative Risk of Resistance

Fungicides labeled for use on pecan fall into several different general chemical classes with different modes of action and associated risks of resistance (Table 1).

Benzimidazoles. Benomyl (Benlate) and thiophanate methyl (Topsin-M) are members of the benzimidazole class of fungicides. These systemic compounds interfere with cell division in the fungus by site-specific inhibition of tubulin biosynthesis (Davidse 1973). A high degree of risk of resistance is associated with the benzimidazoles, due to the very site-specific mode of action of these fungicides and the single-gene control of resistance. Indeed, widespread use during the late 1960's and early 1970's lead to a very rapid development of resistance in a wide variety of fungal pathogens. Benomyl resistance in the pecan scab fungus was first detected in 1975 after only three years of effective control (Littrell 1976).

Table 1. Major fungicides labeled for use on pecan.

Fungicide Class	Compound	Relative Risk of Resistance
benzimidazoles	benomyl (Benlate)	high
	thiophanate methyl (Topsin-M)	high
DMIs	propiconazole (Orbit)	moderate
	fenbuconazole (Enable)	moderate
strobilurins	azoxystrobin (Abound)	moderate
other	fentin hydroxide (Super Tin)	low
	ziram (Ziram)	low
	dodine (Syllit)	moderate

Demethylation inhibitors (DMIs). Propiconazole (Orbit) and fenbuconazole (Enable) are members of the triazoles, the largest of three groups of fungicidal compounds that comprise the C-14 demethylation inhibitors (DMIs), which together with the morpholines, are referred to as sterol biosynthesis inhibitors (SBIs)(Köller 1992). These systemic compounds prevent fungal growth by site-specific inhibition of ergosterol biosynthesis. The DMIs are very effective for control of diseases on a wide variety of crops, including pecan scab. Propiconazole and fenbuconazole are particularly effective during the early part of the season (prepollination). They also provide an additional benefit of providing some eradicant activity by halting development of the fungus when applied within a limited time after infection has occurred (Reilly and Wood 1997). However, because of the site-specific mode of action of the DMIs, a moderate risk of resistance is associated with these highly effective compounds. Although resistance to DMIs has not been reported in the pecan scab fungus, resistance has been documented in many important fungal plant pathogens (Russell 1995). Unlike resistance to benzimidazoles, resistance to DMIs appears to come about gradually, by means of directional selection.

Strobilurins. Azoxystrobin (Abound), the newest fungicide labeled for scab control on pecan, is a member of the strobilurin fungicides. This group of fungicides is comprised of synthetic derivatives of strobilurin A, a naturally fungicidal compound produced by the basidiomycete fungus *Strobilurus tenacellus* (Godwin et al. 1992). The strobilurins have an unusually broad spectrum of activity and prevent fungal growth through inhibition of cellular respiration by blocking electron transport in mitochondria. Like most of the modern synthetic fungicides, the mode of action of the strobilurins is very site-specific and is associated with a moderate risk of resistance, similar to that of the DMIs. Although it has been shown to be highly efficacious against the pecan scab fungus, azoxystrobin has only recently become available and has not yet been very widely used on pecans. Resistant strains of fungal pathogens have been artificially induced in the laboratory (Ziogas et al. 1997), but no field resistance to the strobilurins has been reported.

Others. Fentin hydroxide (Super Tin), ziram (Ziram), and dodine (Syllit) are among the oldest of the fungicides currently available for scab control. These compounds are non-systemic in nature and most effective when applied as protectants. These compounds all have a non-specific, multi-site mode of action. With the exception of dodine, these fungicides are considered to be associated with a relatively low risk of resistance. Despite its non-specific mode of action, a relatively moderate risk of resistance may be associated with dodine. Field resistance to dodine has been reported in a number of pathogens, including the apple scab fungus, *Venturia inaequalis*, a close relative of *C. caryigenum* (Szkolnik and Gilpatrick 1969, Jones and Walker 1976).

Implementation of Resistance Management in Pecan Disease Control Programs

The current recommendations for resistance management in pecan disease control programs are aimed primarily at reducing the risk of development of resistance to the DMIs. However, with the recent addition of azoxystrobin and the likelihood of registration of other strobilurins on pecans in the near future, consideration must be given to management of resistance to strobilurins as well as DMIs. Unlike the DMIs, which are used extensively for pecan disease control, benomyl, thiophanate methyl, and dodine make up a relatively small proportion of the fungicides that are used on pecans. However, because these fungicides are also considered at-risk, they should always be applied in accordance with resistance management guidelines.

DMIs. Management of DMI resistance in pecans is currently based on three approaches: reducing the number of applications per season (or total amount of material), applying as a reduced rate tank-mix combination with fentin hydroxide, or alternation with fentin hydroxide or other chemically unrelated fungicides. Propiconazole is currently available for use on pecan only in a pre-packaged combination with fentin hydroxide, and must be applied as a tank-mix with a limit of eight applications per season. The DMI fenbuconazole (Enable) is still available as a stand-alone compound for pecan disease control and the total amount of material applied per season is limited to 48 oz. per acre. To delay development of DMI resistance, fenbuconazole can be applied either in alternating single or block sprays or full season as a reduced-rate tank-mix with fentin hydroxide. Sensitivities of isolates of *C. caryigenum* to propiconazole and fenbuconazole have been found to be highly correlated (Reynolds et al. 1997). Therefore, these two fungicides should never be alternated or tank-mixed. If fenbuconazole is used alone during the early part of the season, tank mixes of fenbuconazole or propiconazole later in the season should be avoided in order to minimize selection pressure on the pathogen population. Post-infection application of reduced-rate mixtures of DMIs and fentin hydroxide should be avoided due to insufficient post-infection activity of fentin hydroxide (Reilly and Wood 1997).

Strobilurins. Because of its single-site mode of action and moderate resistance risk, azoxystrobin should be applied in accordance with general resistance management principles. Current label restrictions limit the total amount of fungicide to 1.2 lb a.i. per acre per season in order to reduce exposure to the fungicide and selection pressure on the pathogen population. And as with other at-risk compounds, exclusive use of the strobilurins full season should be avoided. Applications of azoxystrobin should be alternated either as single applications or blocks of two consecutive applications with an unrelated fungicide partner, preferably fentin hydroxide because of its non-specific mode of action and low risk of resistance. Reduced rate tank-mix combinations of azoxystrobin and fentin hydroxide, or other unrelated fungicides may also effectively delay development of resistance to the strobilurins. However, there is insufficient data at this time to support the use of tank-mix combinations of azoxystrobin and further experimentation will be necessary to evaluate the effectiveness of various fungicide combinations.

Benzimidazoles. Benomyl and thiophanate methyl have not been recommended as stand-alone fungicides since benomyl resistance was reported in the pecan scab fungus in the mid-1970s. Although benzimidazoles are no longer recommended for scab control, they are still considered to be effective for control of zonate leafspot and powdery mildew. Management of benzimidazole resistance in these pathogens is based on limiting the number of applications, avoiding consecutive applications, and always incorporating fentin hydroxide at reduced rates in tank mixes (Ellis et al. 1998). Because cross-resistance is common among members of this fungicide group, both benomyl and thiophanate methyl can be considered identical with respect to resistance management. Therefore, an application of benomyl followed by an application of thiophanate methyl would be considered a consecutive application and would not be appropriate for resistance management.

Dodine. Although dodine resistance has not been reported in the pecan scab fungus, it is considered an at-risk fungicide and should never be used for full season disease control. Applications of dodine should be alternated with other non-related fungicides, preferably fentin hydroxide. Reduced rate tank-mix combinations with fentin hydroxide or other fungicides may also be effective for resistance management, but additional experimentation will be necessary before such tank-mix combinations can be recommended.

Assessing the Effectiveness of Resistance Management Programs

Fungicide sensitivity monitoring is an important component of a successful resistance management program. Data collected from sensitivity assays of fungal isolates sampled from treated orchards can be valuable for detecting shifts in the sensitivity distribution of a pathogen population before disease control is compromised. Sensitivity monitoring is also an important tool for assessing the effectiveness of resistant management programs. Fungicide sensitivity measurements are relative, and detection of resistant strains or populations must be based on a comparison with "wild-type" sensitive strains or populations. Hence, an essential first step in developing a monitoring program is the establishment of a so-called "baseline" sensitivity distribution for a specific pathogen-fungicide combination. Such baselines have been established for sensitivity of the pecan scab fungus to the DMIs propiconazole and fenbuconazole (Reynolds et al. 1997).

Sensitivity assays are usually based on traditional biological methods such as mycelial growth tests or spore germination tests in liquid or solid media amended with a range of concentrations of fungicide, or using in vivo assays conducted on inoculated host plants or leaf discs (Ishii 1995). In general, these biological assays are quite laborious and timeconsuming. The availability of a more rapid and efficient sensitivity assay would allow a much faster response time in the event of an imminent fungicide control failure. More rapid assays, based on DNA probe technology have been developed for detection of benzimidazole-resistant fungi (Ishii 1995). However this approach is only feasible if the molecular mechanism of pathogen resistance is well understood.

Future Outlook

Fungicides. Additional DMI and strobilurin compounds are currently under development for use on pecan. Cross-resistance between DMIs is common, therefore any new DMI fungicides registered for use on pecan in the future must be used with care to avoid resistance problems. The potential for cross-resistance between azoxystrobin and kresoxim-methyl, a new strobilurin fungicide currently under development, is unknown and must be evaluated before resistance management recommendations can be made (Ammerman et al. 1992). A new pecan fungicide with a non-specific mode of action and low risk of resistance would be highly desirable as a mixing partner for management of resistance to the DMIs and strobilurins. However, fungicides that are currently under development are more likely to be highly specific in their mode of action and at risk for development of resistance. An alternative would be to identify potential mixing partners with a completely different site-specific mode of action from those fungicide classes already in use. Changes in the availability of fungicides for disease control and resistance management also may occur through loss of registration of existing fungicides. This is a concern especially for older, more toxic fungicides such as fentin hydroxide. Loss of mixing partners with a non-specific mode of action and low risk of resistance could have a significant impact on resistance management programs for pecan.

Disease Management. Scab is likely to continue to be major target of fungicide application programs for pecan, especially in the southeastern states. Development of new approaches to scab control, based on weather conditions and/or forecasts, may lead to changes in the timing and frequency of fungicide applications that could have a significant impact on resistance management programs (Sparks 1995, Bertrand and Brenneman 1998, Driever et al. 1998).

Resistance management strategies. The relative merits of alternations versus mixtures for resistance management have been debated for many years. There is some evidence, based on theoretical models, that alternations are somewhat more effective than tank-mixes for delaying simultaneous development of multiple resistance to both fungicides (Shaw 1993). However, tank-mix combinations of appropriate fungicide partners, especially when sold exclusively as a pre-packaged combination, do provide a greater level of assurance that the fungicides are applied according to resistance management recommendations. Continued field evaluation of new combinations of fungicides, both in alternation and as tank mixes, is needed in order to provide sound resistance management recommendations.

Resistance Monitoring. Faster, more efficient techniques for sensitivity monitoring are needed to facilitate timely detection or warning of resistance problems and evaluation of resistance management programs. Rapid and efficient biotechnological techniques using DNA probes can be used to detect fungicide resistance once the mechanism of resistance has been determined at a molecular level. This approach has been successful for monitoring resistance to benzimidazoles, for which the genetic mechanisms of resistance are well understood (Ishii 1995). For DMIs, however, the mechanism of resistance in fungal pathogens is complex and poorly understood, making it difficult to utilize biotechnological methods for detection of resistance. The strobilurin fungicides are still so new that there is very little information available about the mechanisms of resistance. Much more research will be needed to elucidate the mechanisms and genetic control of resistance before application of this new technology can be fully exploited.

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