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# Optimizing Fungicide Applications for Plant Disease Management: Case Studies on Strawberry and Grape

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## 1. Introduction

Fungicides are important tools for management of plant diseases caused by fungal and oomycete pathogens. Without use of fungicides, major crop losses are inevitable, and food supply networks as we know today are most likely not able to sustain itself. As a result, fungicides are applied in regular basis in many parts of the world; however, their applications need to be optimized in order to obtain the best result in disease management due to multiple factors such as fungicide efficacy, the risk of resistance development, environmental concerns, pesticide residue in harvest, impact on beneficial organisms, etc. The ultimate goal is to keep the losses from diseases to a level that does not represent a threat to the crop production and to the economy of the grower while reducing the number of applications as much as possible. In order to achieve this goal, growers commonly employ integrated pest management (IPM) approaches where multiple management options are used together to achieve best efficacy with minimum chemical usage. Especially in environmentally challenging growing areas, use of fungicides is an important component of the IPM approach. Abusive uses of fungicides can cost not only growers' budget, but also cost society and environment. Therefore, fungicide usages need to be carefully planned with a good understanding of plant disease epidemics, their components (host, environment and pathogen), fungicide mode of action (biochemical, biological, physical), risk of resistance development, and host physiology, among other aspects. In this chapter, we will review these components that are involved in decision-making process to optimize fungicide application. The main focus of discussion is on management of diseases of strawberry and grape, because both are high value, intensively managed crops where application of fungicides are conducted on a regular basis.

In both strawberry and grape productions, it is not uncommon to observe an excessive number of fungicide applications, which happens sometimes as a result of the lack of knowledge of the pathogen biology and epidemiology, fungicide mode of action and fungicide residues. Or simply growers do not want take risks because of high costs and values of these crops. Moreover, the availability of several groups and mixtures of fungicides in the market is creating confusion among growers who are constantly in need of learning how to integrate a new chemistry in their plant disease management program. It is further confusing not only to growers but also to educators and researchers as well. Some of new formulations or molecules are simply a mixture of known active ingredients, or a different brand name yet the same active ingredient, or a different chemical name with the same mode of action, or a mixture of known active ingredients with a different percentage, etc.

In some agricultural settings such as the wet areas of the Midwest and Eastern US, tropical and subtropical areas of Central Mexico, the need of fungicide use is continuous during the course of the crop development; therefore it is a challenge for growers to keep their fungicide program season after season. Although it is not always considered, there are many factors that influence the decision making process of a grower to apply fungicides. If you put in a simple sense, what a grower wants is to manage a population of pathogens at the end of the day; however at the same time, he/she needs to be aware of the existence of the right tools that provide her/him an economical, effective, and sustainable (in both economic and environmental sense) solution. In addition, because of social pressure against the use of chemical in agriculture, fungicides applications for plant disease management need to be carefully selected. Since development of any plant disease is a result of a complex interaction among host, pathogen, environment, and sometimes a vector of the pathogen, the optimization of a fungicide application program should be based on the knowledge of disease dynamics, fungicide and mode of action in relation to development of epidemics (Madden 2006; Madden et al. 2007). In order to establish season-long programs to manage key diseases, growers need to learn and understand knowledge of information related to the factors that affect the efficacy of a fungicide, the biology and epidemiology of the disease, and crop physiology and the environment.

In this chapter, we explore the factors that growers, consultants, and researchers need to consider in order to establish optimized season-long programs with ecologically and economically sound approaches. We describe different components that influence the development of epidemics and their impact on crop disease management and the whole season approach to manage diseases, disease epidemics, fungicide resistant and its management, integrated pest management, and uses of disease risk assessment tools. In addition, we present two case studies managing diseases using fungicides based on information considering different tactics and strategies to reduce the number of fungicide application, and risk resistance development on grape and strawberry.

## 2. Components of epidemics and fungicides

Plant diseases are the result of the interaction among the host, the pathogen and the environment. Plant pathologists often describe this relationship, or model, as a plant disease triangle (Francl 2001; Agrios 2005). Each component of the disease triangle plays an important role on the development of diseases. When there is a compatible interaction between a host and a pathogen (i.e., a pathogen can cause disease on a host), the environment is the element that triggers development of a plant disease. Thus, a basic idea of plant disease management is to break the disease triangle from forming by understanding the role of each element. For instance, planting a disease resistant variety is a way to disturb the disease triangle by eliminating the host so that the triangle cannot be formed.

When we consider the change of plant diseases over time and space, we are dealing with plant disease epidemics (Madden 2006; Madden et al. 2007). Since time is another factor added to the triangle, some use a modified disease triangle, which becomes a tetrahedron (Francl 2001). Sometimes it is a challenging task for agricultural educators (such as crop specialists) to describe the concept because it deals with another dimension (time). However, it is important to inform growers that the disease you see today is a consequence of an infection that happened a certain time ago, or even a consequence of multiple infections that happened over the course of time.

Since we are dealing with the progress of disease(s) over time, we need to understand the life cycle (often referred as a disease cycle) of pathogens, which are divided into two groups, monocyclic (one disease cycle per season) and polycyclic (multiple disease cycles per season). Based on the disease cycle, management strategies can differ. For example, one of strategies of plant disease management can involve elimination or reduction of the amount of primary inoculum, which reduces the rate of infection by reducing the probability of pathogens to find healthy host tissues and/or by limiting the time the pathogen and host populations interact (Nutter 2007). In some monocyclic disease cases, only one application of fungicide might be needed. For example *Fusarium graminearum*, a causal agent of Fusarium head blight of wheat causes infection on kernels during anthesis, therefore, protection of wheat during this stage of development is the key for the management of this disease (Nita et al. 2005).

On the other hand, when a continuous release of pathogen inoculum is occurring and host tissues are susceptible over time, multiple applications might be needed. In order to deal with polycyclic diseases, often several applications are needed to delay the onset of the epidemic. In this case, the impact of fungicide applications will be on the rate of the epidemic by reducing the probability of successful infection and/or successful completion of life cycle (= production of spores) (Fry 1982). Early studies by J. E. Van der Plank (1963) introduced many of these concepts, and it was followed by many plant disease epidemiologists who utilized these concepts to develop plant disease models and management strategies such as a use of disease risk assessment (forecasting or warning) tools (Zadoks and Schein 1979; Zadoks 1984; Madden et al. 2007). Some of disease risk assessment tools aim to determine the critical time when the disease become a threat and/or have an economic impact. Disease risk assessment tools can be very useful to reduce the costs of disease control and increase safety of the produce by helping growers to use fungicides in a timely and more efficient manner (Zadoks 1984; Hardwick 2006; Madden et al. 2007).

## 3. Fungicide resistance and plant disease management

When we discuss about fungal disease management, discussions on the issue of fungicide resistance cannot be avoided. Development of fungicide resistance fungal isolates was documented as early as 1960's when *Penicillium* spp. on citrus (citrus storage rot) was found to be resistant against Aromatic hydrocarbons (Eckert 1981). The other examples from that decade are resistance to organomercurials by cereal leaf spot and strip caused by

*Pyrenophora* spp., dodine resistant apple scab (*Venturia inequalis*), and QoI (**Q**uinone-**O**utside **I**nhibitor) resistance against grape powdery mildew (caused by *Erysiphe necator*) in the field in Europe, and North America in 1990's to 2000's (Staub 1991; Baudoin et al. 2008).

Fungicide resistance develops when a working mode of action loses its efficacy against target fungal pathogen. When fungicide resistance appears in the field, it is often the case that a particular fungicide (or a mode of action), has been used for a several years or seasons, and growers find that the efficacy of that fungicide has been noticeably reduced or even lost. This type of resistance is often called 'field resistance' or 'practical resistance' in contrast to the cases when the resistance isolate is found only in the laboratory conditions (= laboratory resistance) (Staub 1991). Some of laboratory resistance isolates can only survive under protected conditions because they are not adequately fit to compete and survive in the field thus, the presence of these laboratory resistant isolate may or may not be a threat to the real world. Attempts were made to predict the development of field resistance based on populations of laboratory resistance isolate; however, it has been difficult. For example, although the presence of resistant isolates of *Botrytis* against dicarboximides was found in laboratory, the development of field resistance was slower than expected (Leroux et al. 1988).

The resistant mechanisms, whether a single gene or multi-locus function, maybe present naturally among wild population in a small quantity and a repeated application of a particular mode of action select these rare populations to thrive. In some cases, the development of fungicide resistance appears to be a sudden event. This type of resistance is also called 'qualitative', 'single-step', or 'discrete' resistance (Brent and Hollomon 2007). This qualitative resistance tends to appear relatively soon after the introduction of the compromised mode of action and stay once appeared. One of examples would be benzimidazole resistance of apple scab pathogen (*Venturia inequalis*) where resistant isolates appeared only after two seasons of benzimidazole fungicide application (Shabi et al. 1983; Staub 1991). In some cases, a gradual recovery of sensitivity can happen; however, as soon as an application of the compromised mode of action resumes, the resistance tends to come back quickly as in the example of potato late blight pathogen (*Phytophthora infestans*) to phenylamide fungicides (Gisi and Cohen 1996).

In the other cases, development of fungicide resistance is gradual. This type of resistance is called 'quantitative', 'multi-step' or 'continuous' (Brent and Hollomon 2007). Examples of quantitative resistance are the cases of many fungal pathogens to the DMI (sterol **D**e**M**ethylation **I**nhbitors) where the reduction of efficacy can be observed over several years or seasons (Staub 1991). For quantitative resistance, reduced use of fungicides of the same mode of action tends to revert populations back to more sensitive state. This decline of the resistance could be due to incomplete resistance, or lack of fitness, or both (Staub 1991).

Another concern on the resistance is the phenomenon called 'cross-resistance' where resistance to one fungicide translates into resistant to other fungicides, which are affected by the same gene mutation(s). Often time it happens with fungicides that are different in chemical composition, while share the same mode of action. One example is the case of benzimidazole fungicide resistance where pathogen strains that resist benomyl were resistance to carbendazim, thiophanate-methyl, or thiabendazole (Brent and Hollomon 2007). Moreover, in some cases, a fungal strain can be resistant to two or more different mode of action or acquire 'multiple resistance'. For example, *Botrytis cinerea* a causal agent of bunch rot of grape and many other plant diseases, is commonly resistant to both benzimidazole and dicarboximide fungicides (Elad et al. 1992).

As noted previously, repeated application of the same mode of action often increase the risk of development of fungicide resistant population. Because of that, intensively managed agricultural crops, such as wine grapes and strawberries, the risk of fungicide resistance development is higher due to frequent application of fungicides throughout a season. For example, in the eastern US grape growing regions, wine grape growers apply more than 10 applications of fungicide year after year (Wolf 2008), but in other regions such as the Central part of Mexico, more than 20 fungicide applications can be done on strawberries in a growing season. Once fungicide resistance is developed against a certain mode of action, it is not only a loss for growers, but also a huge loss to chemical companies that invested a considerable amount of money and time to develop the product. Currently, there are more than 150 different fungicidal compounds used worldwide (Brent and Hollomon 2007). The total sales of fungicide are estimated to be $7.4 billion in US dollars, and grapes are one of the largest consumers of fungicides.

## 4. Management of fungicide resistance

There are several tactics to reduce the risk of fungicide resistance development. Common approaches implemented from fungicide manufacturers and regulatory agencies are 1) set a limit on the number of application per year, and 2) production of a pre-mixed material. The aim of setting a limitation or a cap on the number of applications per season is to reduce the rate of shifting from sensitive to resistance population by providing a gap between usages. If fungicide sensitive population is less fit than sensitive population, then the interval will provide a time for sensitive population to take over the resistant population. However, in some cases, the cap on the usage did not help the development of fungicide resistance. For example, Baudoin et al. (2008) found that although growers were following a '3 times per season' cap set by an international organization, Fungicide Resistance Action Committee (FRAC), QoI (e.g. strobirulins) resistant grape powdery mildew appeared in the field after 10-15 applications over several years of use. It seems that the 'cost' of having QoI fungicide resistance (i.e., *G143A* mitochondorial cytochrome b gene) does not affect the fitness of the fungus. On the other hand, the cap approach could help reducing the risk of DMI resistant isolates since fungal population seems to revert back to be sensitive when DMI is not used in the field (Staub 1991; Brent and Hollomon 2007).

The aim for pre-mixed material is to create a mixture of fungicides with multiple modes of action. There is evidence of reduced rate of fungicide resistance by mixing two (or more) different mode of action. For instance, Stott et al. (1990) compared the population shift of barley powdery mildew (caused by *Erysiphe graminis*) and showed that DMI and ethirimol sensitive populations did not shift to resistant population when both materials were used together. This approach seems to be favored by fungicide manufacturing companies; however, as we noted earlier, these pre-mixed materials can cause confusion among growers especially when seemingly new materials were combinations of previously introduced modes of action in reality.

When a host crop requires intensive disease management, the aforementioned two approaches may not be enough to effectively manage the development of fungicide resistance. For instance, in order to manage grape powdery mildew under eastern US growing conditions, wine grape growers typically use a fungicide (or multiple fungicides) for powdery mildew practically every time they spray (10-15 times per year). If they use a DMI early in the season, they may not have much choice later. Thus, careful planning and execution of plant disease management becomes very important. In order to achieve the goal, many successful growers extensively practice the Integrated Pest Management (IPM) or Integrated Plant Disease Management (IPDM) approach.

## 5. IPM approaches revisited

A basic concept of IPM is to combine multiple approaches of disease management in order to achieve the best result (Agrios 2005). These approaches are 1) cultural control, 2) use of genetic resistance, 3) biological control, and 4) chemical control. In the case of grape disease management, cultural practice can include (but not limited to) site selection, proper nutrition management, selective pruning of dormant canes, canopy management (shoot training, leaf removal, etc), etc. Genetic resistance can be introduced by selecting disease resistant varieties such as some of French hybrids. Often time the challenge is to select resistant varieties with high market demands. One of success stories of such a case is variety called 'Norton'. This highly disease resistant variety for wine making has gained popularity in the Eastern US grape growing regions since 1990's (Ambers and Ambers 2004). There are several biological agents available for use in grape production; however, none of them seems to produce reproducible results. It is partly due to the fact that growers want to use them as if they were using chemical options. Chemical management approaches should be considered only after these non-chemical approaches are considered. Integration of these approaches not only increases the efficacy of overall management strategies, but also, can reduce the monetary cost associated with chemical management approach (e.g., costs for purchasing chemicals, labor and fuel to apply chemicals, etc).

Even after other IPM approaches are considered, growers often need to resort to chemical management options because of environmental conditions that highly favor disease development. There are a few more items to be considered before application of fungicide in order to increase the efficacy. First of all, growers need to identify target pathogen(s) correctly. Then, growers need to select the best tool for management of the target pathogen(s) based on the situation at hand. In order to guide this complicated decision making process, it is necessary to have a better understanding of pathogen and host biology, as well as awareness on legal requirements.

As with any other pest management, identification of the target organism is a very critical component of plant disease management. For instance, symptoms of downy mildew of grape (caused by *Plasmopara viticola*) and powdery mildew of grape (caused by *Erisyphae necator*) may look similar to untrained eyes; however, materials for downy mildew are most likely not effective against powdery mildew, and *vise versa*. Thus, misidentification of disease symptoms can result in unnecessary application of fungicides.

After correct identification of the target disease(s), growers need to determine the best tool(s) for the situation at hand. Both host crop physiology and pathogen population changes throughout the course of the season, and these changes can influence disease triangle of the target pathogen. As we covered in the previous section, in order for a pathogen to successfully infect a host crop, a susceptible host, a pathogenic pathogen, and a disease-conducive environment have to be present at the same time. However, a pathogen may not produce spores at a right timing or a host may not be susceptible at a certain time of its lifecycle. Even if there are spores and hosts are susceptible, if the environmental conditions are not conducive for infection, disease cannot be developed. Thus, it is very important to understand both pathogen's and host's lifecycles, as well as environmental conditions for infection, so that growers can place fungicide application to efficiently disrupt the formation of the disease triangle without wasting their effort.

Changes in host physiology throughout the season, especially fruit development, can be a key factor to determine when and how fungicide should be applied. For example, it is important to protect flowers of strawberry from Botrytis infection because flower infection result in latent infection on berries later in the season (Mertely et al. 2002). Results from Merteley et al., (2002) indicate that Botrytis fruit rot can be controlled with an application of fenhexamid when applied at anthesis. They were also able to relate a linear regression equation between time of application and Botrytis fruit rot incidence, which can guide growers to adjust their spray timings. Legard et al. (2005) integrated information of the crop physiology, epidemiological information and fungicide efficacy to develop reduced fungicides programs to control Botrytis fruit rot in Florida. Their results indicate that in the early stage of the season low rates of captan were as effective as high rates for disease control, and later in the season the control was significantly improved by applications of fenhexamid at the second bloom peak period. In the case of grape production, ontogenic resistance has been well documented against many of major pathogens such as black rot (Hoffman al. 2004), powdery mildew (Ficke et al. 2002; Gadoury et al. 2003), and downy mildew (Kennelly et al. 2005). Grape berries become resistance against downy mildew, powdery mildew, and black rot approximately 4-5 weeks and 3-4 weeks after bloom for French and for American varieties, respectively. By knowing this information, growers can concentrate their effort to protect berries during this critical period.

In addition to biological factors, legal or legislative factors can influence fungicide application decision-making process. For instance, a product containing mancozeb has a 66-day PHI (pre-harvest interval) set by the EPA (Environmental Protection Agency) for an application on grape in the US. Thus, grape growers need to adjust their spray program against downy mildew or black rot when they are expecting to harvest within 2 months. Also, REI (re-entry interval) can be a limiting factor. A product Topsin-M (thiophanate-methyl) has a REI of 2-days for grapes, and a product Pristine (boscalid + pyraclostrobin) has a warning on the label that growers cannot work on grape canes within 5 days after application. Thus, it is difficult to use either Topsin-M or Pristine when constant canopy management is required. The other factors can influence fungicide application is an incompatibility issue. For example, several fungicides, including chlorothalonil can cause phytotoxicity on 'Concord' and related American grape varieties (Goffinet and Pearson 1991).

## 6. Physical mode of action of fungicides

There is yet another factor to be considered prior to an application of fungicide, that is, physical mode of action of fungicide. Physical mode of action (PMoA) describes the effect a fungicide with respect to the time of placement of a fungicide in relation to the host-pathogen interaction, that is on pre-infection, post-infection, pre- and post-symptom, and vapor activity (Szkolnik 1981; Wong and Wilcox 2001) and the duration and degree of the fungicides activity (Pfender 2006).

PMoA of protectant fungicides is pre-infection effect. It can reduce the infection efficiency as a result of the placement of a fungicidal material on plant tissues. McKenzie et al. (2009) found that applying captan 2 days before inoculation on strawberry crown rot (caused by *Colletotrichum gloeosporioides*), disease intensity was consistently reduced at the end of the season. Azoxystrobin, pyraclostrobin and thiophanate-methyl performed better if applied 1 day after inoculation, but their effect reducing the disease was variable. Based on these results the recommendation was to spray captan throughout the season in a protectant strategy, and azoxystrobin, pyraclostrobin and thiophanate-methyl if an infection event was present in order to keep the disease at low levels.

On the other hand, systemic fungicides with more curative (eradicating) activity can impact the processes of infection and establishment by pathogen, thus these are post-infection and can be pre- or post-symptom effect. Vapor activity can facilitate pre- and post-symptom effects. A single fungicide can provide both protectant and curative activities. For example, fungicides such as strobilurins (QoI) will mainly impact on spore germination, as they interfere with mitochondrial respiration (Bartlett et al. 2002), giving an excellent protectant activity. At the same time, the QoI can provide good curative activity against rusts such as *Puccinia hemerocallidis* and *Puccinia graminis* subsp *graminicola* (Godwin et al. 1992; Pfender 2006). In some cases such as *Cercospora beticola*, that causes Cercospora diseases on sugarbeet, good post symptom activity (eradicant) an antisporulant activity of this group of fungicides has been reported (Ypema and Gold 1999; Anesiadis et al. 2003). In other cases such as downy mildew of grape, caused by *Plasmopara viticola* (Wong and Wilcox 2001) and *Phytophthora cactorum* on strawberries (Rebollar-Alviter et al 2007) these fungicides provide good protectant activities, but do not perform well in post-infection treatments. Other groups inhibiting the sterol biosynthesis (SBI/DMI) do not have direct effect on spore germination, but impact more directly on mycelial growth. Hoffman et al. (2004) found that a DMI, myclobutanil, provides a better post-infection activity against black rot of grape, compared with azoxystrobin, which provided a slight evidence of a post-infection activity.

## 7. Fungicide use based on disease risk assessment tools

Now we have covered basics of plant disease development, management approaches, fungicide resistant issues, and physical model of action, the next step is to combine these together. As we briefly touched earlier, one of approaches taken by many researchers and growers are the use of disease risk assessment (or forecasting or warning) tools to minimize the use of fungicides by determining the best timing for application. There are several examples of risk assessment tools used together with the knowledge of the physical mode of action of fungicides. For example, Madden et al. (2000) evaluated an electronic warning system for downy mildew based on infection of leaves of American grapes, *Vitis labrusca*, productions of sporangia and sporangial survival over a period of 7 years. Sprayings were done when the system indicated that environmental conditions were favorable for sporangia production. Their results indicated that during this time the use of the warning system reduced the number of applications of metalaxyl plus mancozeb from one to six applications compared to the standard calendar based program. Wong and Wilcox (2001) evaluated the physical mode of action of azoxystrobin, mancozeb and metalaxyl against *Plasmopara viticola*, the causal agent of grape downy mildew. Azoxystrobin was effective in pre-infection treatments, but was ineffective when applied as a post-infection treatment. However, good effect was observed on reduction of sporulation, and reduction lesion size in post-symptom applications. Mancozeb was also excellent protectant but did not have any effect on post infection applications. Metalaxyl provided good pre-infection, post-infection and eradicant activity. Kennely et al. (2005) indicated that mefenoxam has strong vapor activity against *Plasmopara viticola*, grapevine downy mildew and 48 h of systemic activity in post-infection applications. Caffi et al. (2010) evaluated a warning system to control primary infections of downy mildew on grapevine, and results indicated that the number of applications can be reduced by more than 50% with significant savings in cost per ha without compromising downy mildew control.

Working with anthracnose fruit rot of strawberry, Turecheck et al. (2006) evaluated the pre- and post-infection activity of pyraclostrobin on the incidence of anthracnose fruit rot at different times of wetness periods and temperatures. Results indicated that pyraclostrobin was less effective when applied in post-infection with the longest wetness duration (12 and 24) and high temperature (22 and 30 C). The post-infection application had a significant effect when applications were made within 3 and 8 h after the wetness period. Under field conditions, applications made after 24 h after an infection event were able to successfully control the disease, indicating the possibility to incorporate pyraclostrobin in a disease management program in strawberry in a curative form if infection events occurred in the previous 24 h. In a similar study, Peres et al. (2010) indicated that anthracnose fruit rot was effectively controlled with captan on pre-infection under short wetting period and fludioxonil + ciprodinil was effective when applied in pre-inoculation, but also when applied at 4, 8, and 12 h after inoculation, but the efficacy was higher under short wetting periods (6 o 8 h). These studies indicate that performance of fungicide is strongly influenced by wetness duration regardless of the ability of the fungicide move in plant tissues.

Thus, growers face multiple layers of factors such as host-pathogen dynamics, fungicide resistance, physical and biochemical mode of action, IPM strategies, etc. in order to make decisions on fungicide application. Also, note that we were focus only on biological considerations, but not covering many of social and environmental factors such as society's concerns on fungicide use, issues on waste water management, and so on. In addition, there is a whole art and science of fungicide application technologies that is beyond our scope of this chapter. Instead of widening our topics, we would like to focus on the factors we discussed in this chapter by presenting two case studies that are compilations' of multiple studies to establish an optimal use of fungicide(s).

## 8. Case study 1: Phomopsis cane blight and leaf spot of grape

A series of studies by Nita et al. (2006a; 2006b; 2007a; 2007b; 2008) showed a multi-prong approach to develop a sound management strategy against Phomopsis cane and leaf spot of grape. Phomopsis cane and leaf spot is a common disease of grape in the U.S. and other grape growing regions around the world (Pearson and Goheen 1988; Pscheidt and Pearson 1989). The fungus, *Phomopsis viticola* (Sacc.), is the causal agent of the disease (Pearson and Goheen 1988). Typical symptoms on leaves are yellow spots, which varies in size (less than 1 mm to a few mm). On canes and rachis, it causes necrotic lesions that can be expanded to cause canker. The infected tissues become weak and prone to be damaged by wind. With heavy infection on rachis, fruit drop can be observed. Infections on fruits cause a fruit rot and thus directly decrease yield and fruit quality. Up to 30% loss of the crop has been reported in the Southern Ohio grape growing regions (Erincik, et al. 2001).

The source of inoculum in a given season consists of canes or trunks that were infected during previous growing seasons (Pscheidt and Pearson 1989). The fungus survives in the infected tissues over the winter, and in the spring, numerous pycnidia arise on infected canes. Conidia from these pycnidia are splashed by rain onto new growth (i.e., canes, leaves, and clusters) to cause infection. According to previous studies, *P. viticola* can be active in relatively cool weather conditions (7-8 C) (Erincik et al. 2003). Since they do not produce new spores during the season, it is considered a monocyclic disease.

In order to evaluate efficacy of currently available fungicides, Nita et al. (2007a) examined several fungicides for their protectant and potential curative activity against Phomopsis cane and leaf spot of grape. Fungicides with variety of mode of action, strobilurin, thiophanate-methyl (benzomidazole), myclobutanil (DMI), EBDC (mancozeb), calcium polysulfide (lime sulfur), were tested as protectant as well as curative application in a controlled environment study. Protectant application was applied a few hours prior to an artificial inoculation of leaves and shoots using spore suspension water that contained 5 x $10^6$ spores per ml. Various patterns of post-inoculation (curative) application were tested. The shortest period between inoculation and application of a fungicide was 4 hours and the longest was 48 hours. In addition, a treatment with or without an adjuvant (product name Regulaid or JMS Stylet Oil) was also tested. These adjuvants were added in a hope that it might help facilitate movement of chemical into tissues. In addition, up to 150% of labeled rate of fungicide was examined to see a potential dose effect. Results indicated that all materials tested, regardless of a higher rate and/or a presence of adjuvant, did not show evidence of curative activity. On the other hand, strobilurin, calcium polysulfide, and EBDC showed a good protectant activity, up to >85% disease control [(treatment disease severity-negative (=untreated) control disease intensity)/negative control disease intensity], indicating that the management strategy for Phomopsis cane and leaf spot has to focus on protection of vines.

Then the same group evaluated the effect of dormant season fungicide applications of copper and calcium polysulfide against Phomopsis cane and leaf spot of grape disease intensity and inoculum production (Nita et al. 2006a). These dormant season fungicide applications aimed to reduce the source of inoculum by disturbing fungal colonies surviving on grape trunk tissues. Results indicated that fall and spring and spring applications of calcium polysulfide (10% in volume) provided 12 to 88% reduction in disease intensity and inoculum production. Thus, the reduction of disease intensity was not sufficient. Although inoculum production (the number of pycnidium per square cm) was significantly reduced, none of tested canes had zero pycnidium, indicating that there will be a plenty of inoculum available even with the best treatment. In the same study, the authors examined calendar-based applications of mancozeb or calcium polysulfide (0.5% in volume), which reduced 47 to 100% disease incidence and severity. The result indicated that although sanitation approach against this disease did not provide reasonable reduction in disease development, early season applications of a protectant fungicide (mancozeb or calcium polysulfide) provided a better efficacy. These results confirmed previously discussed management recommendations (Pearson and Goheen 1988; Pscheidt and Pearson 1989).

Nita et al. (2006b) also evaluated a warning system (based on temperature and wetness duration following rain) for Phomopsis cane and leaf spot of grape by applying fungicides based on prediction of infection events considering three criteria for risk: light, moderate and high. The infection condition was determined previously by Erincik et al. 2003. This study was conducted to determine if the warning system would provide a reasonable disease control compared with a calendar-based, 7-day interval protectant fungicide application. The warning-system based approach resulted in two to three times less number of applications while the percentage of control was often not significantly lower than the 7-day protectant schedule based on mancozeb, which constantly provided 70-80% and over 95% disease incidence and severity control, respectively.

The same group expanded this study by examining Phomopsis cane and leaf spot disease survey data using various statistical tools and modeling approach (Nita et al. 2007b; Nita et al. 2008). They found out that the variation of disease incidence observed in 20 different commercial vineyard locations over three consecutive years could be explained by a combination of local weather conditions and fungicide application trends. They further found that growers who had a better early season fungicide program (i.e., a use of dormant application of lime sulfur and/or mancozeb application soon after bud break) tended to have lower disease incidence than others who did not protect their vines during that time.

These series of studies showed that pre-season dormant application does not provide satisfactory reduction of this disease, and there are no potential curative materials; however, a dormant season application can be used in a conjunction with early season protectant fungicide applications, a warning system approach can be a good tool to be used, and more importantly, protection of grape tissues during early part of the season was found to be critical for the management of Phomopsis cane and leaf spot of grape. The Eastern and Midwestern US grape growing regions often receive a series of rains in April to May when new grape shoots are emerging, and pathogen can infect tissues under relatively low temperatures conditions, 7-8 C (Erincik et al. 2003; Nita et al. 2003). Therefore, good protection of newly emerging shoots (when new shoots are about 2.5-7.5 cm in length) using a protectant fungicide is a standard recommendation for this disease (Pscheidt and Pearson 1989; Nita et al. 2007b).

## 9. Case study 2: Leather rot of strawberry

Crown and root rots, such as those caused by *Colletotrichum* spp, *Phytophthora* spp. and *Verticillium* spp., and fruit rots, such as *Botrytis cinerea*, *Colletotrichum acutatum*, and *Phytophthora cactorum* are among the most important pathogens causing disease on strawberry that cause more losses around the world.

Leather rot caused by *P. cactorum* is one of most common disease on strawberry, especially in systems such as matted row and annual systems. The disease is less severe and not very frequent in high tunnel system, mainly because plastic tunnels prevent rain to reach plants and induce splash dispersal of the pathogen. On strawberry all stages of fruit development may be infected by this pathogen, including flowers. On green fruits dark areas covering the entire fruit may develop which later appear leathery and eventually mummify. Mature fruits do not always show the typical symptoms, except they appear discolored and whitish in some areas. However, diseased fruits are in general easy to distinguish because the bad off-odor and taste, which is caused by phenolic compounds (Jelen et al. 2005). In Ohio, losses over 50% have reported (Ellis and Grove 1983) and in areas with medium to low technology levels in open field strawberry plantings under annual production systems in countries such as Mexico, the disease can be a problem during the rainy season of the year (June to October) where losses can reach up to 30% of production.

Development of leather rot is favored by excessive wet weather, especially on saturated soils with poor drainage. In this pathosystem, oospores represent the primary inoculum, which is a survival structure. With moisture, oospores germinate to produce sporangia. Sporangia can germinate and produce a germ tube for infection, or can give a rise to zoospores that can swim in water. With a rain event, both sporangia and zoospore are splash dispersed to fruits to cause infection. Once established, new sporangia can form on the infected fruit to cause another infection. Thus, it is considered a polycyclic disease. Extensive studies conducted on the epidemiology of the disease in the past decade have shown that wetness duration and temperature (17 to 25 C) are important factors for disease development. Splashing of zoospores and sporangia is caused by rainfall and wetness periods can be as short as 2 h are sufficient for the oomycete to cause infection (Grove et al 1985a; Grove et al. 1985b; Madden et al. 1991). Typically there is a latent period of 5 days for full development of symptoms.

Management of leather rot is based on the use of fungicides and cultural practices such as avoiding saturated soils by proper site selection, improving soil drainage and applying straw mulches between rows. Applying straw mulch between row spaces prevents fruits from touching the soil and standing water, and reduces the splashing of water droplets containing sporangia and zoospores (Madden et al. 1991). Protective fungicide program using captan and thiram are widely used; however, both fungicides are not able to control the disease when weather conditions favor leather rot development. Therefore fungicide with a different biochemical, and physical mode of action with the ability to penetrate plant tissues need to be used.

In order to select the proper fungicide, the efficacy of fungicides was defined in the field (Rebollar-Alviter et al. 2005). During 2003 and 2004, the efficacy of pyraclostrobin, azoxystrobin, potassium phosphite and mefenoxam was evaluated in Wooster Ohio, USA against leather rot of strawberry grown in a matted row system. Treatments were applied as a preventive application at the initiations of bloom. In order to create conditions that favor leather rot development, straw between the rows was removed and then plots were flooded until water puddle between the rows at different times using an overhead irrigation system. Results from these experiments indicated that during the two years of testing, disease incidence on fruits varied from 58 to 67% in the controls. No significant differences were detected among the fungicides treatments. Disease incidences ranged from 0.3 to 0.5% with the QoI fungicides (azoxystrobin and pyraclostrobin), 0.8 to 5.4% with potassium phosphite, and 0.3 to 11% with mefenoxam (Rebollar-Alviter et al. 2005). Interestingly, these experiments showed that both QoI fungicides tested were highly effective for control of leather rot of strawberry. Thus, these QoI fungicides can be used in a disease management program alternating with potassium phosphite and/or mefenoxam, which are known to be efficacious to control the disease (Ellis et al. 1998).

In order to understand some aspects of the physical mode of action of the QoI, potassium phosphite, and mefenoxam fungicides that were tested in the previous work, a greenhouse study was conducted. Fungicides were applied on pre-infection, 2, 4 and 7 days before inoculation with a zoospore suspension ($10^5$ zoospores/ml) and 13, 24, 36 and 48 h after inoculation. A wetness period of 12 h was applied to plants and fruits either before or after inoculation, and disease incidence was recorded 6 days after inoculation. Results indicated that all fungicides applied in pre-infection provided excellent protection activity against the disease when applied up to 7 days before inoculation. These studies confirmed the protectant activity of all fungicides in previous experiments in strawberry. However, the results when the fungicides were applied in post-inoculation (curative application), both QoI fungicides had some effect 13 h after inoculation reducing disease incidence by 60%. Nevertheless when both fungicides were sprayed 24, 36 and 48 h after inoculation there was no disease control. In contrast, the systemic fungicides potassium phosphite and mefenoxam successfully controlled the disease up to 36 h after inoculation with no significant differences between these two fungicides. At 48 h both fungicides still had some moderate control, but not enough to be considered in a curative strategy for disease management (Rebollar-Alviter et al. 2007a).

These results were then used in conjunction with the previous knowledge on the disease epidemiology in order to evaluate disease management programs and to optimize fungicide application. A 3-year study was conducted in a field to examine efficacy of several modes of action (mefenoxam, phenilamides; azoxystrobin and pyraclostrobin, QoI, and potassium phosphite, phosphonate) against leather rot. In previous studies on a forecasting system for leather rot; occurrence of rain was considered a better indicator of risk of disease development than temperature condition or length of wetness duration (Reynolds et al. 1988; Madden et al. 1991). This is probably because this pathogen requires very short wetness periods (2 h) to infect (Grove et al. 1985a), and it can also infect under a wide range of temperatures. Therefore, specific infection conditions (i.e., temperature or length of wetness duration) would not clearly define the risk conditions. Rather, a detection of individual rainstorm and the amount of rainfall during critical periods is a better indicator for post-infection application of a fungicide. The amount of rainfall is critical because it will be a predictor for the dissemination of the spores to susceptible fruits (Ntahimpera et al. 1998).

Based on previous experiments where post infection activities of mefenoxam and potassium phosphite indicated that this fungicides were able to control the disease up to 36 h after inoculation, and considering that epidemic is basically driven by moderate to heavy rain events (Reynolds et al. 1987; Reynolds et al. 1988), scheduling fungicides after the occurrence of rain events taking in to account fungicide persistence in plant (at least 7 days) and other factors that affect the efficacy of fungicides, as well as weather predictions, it would be possible to reduce the number of applications during the critical time for disease development. These experiments indicated that post infection treatments applied after flooding events were as effective as those applied on a calendar basis, but with 1 to 3 fewer sprayings. One spraying of mefenoxam was sufficient to keep the disease under control when applied within 36 h after a rain event. Similarly, 2 sprayings of potassium phosphite were enough to control the disease when sprayings were done within the same time after the occurrence of a rain event. Whereas in calendar based applications (7 days schedule) four sprayings were necessary to control the disease using programs based on azoxystrobin and potassium phosphite, 1 spraying of mefenoxam and 2 of potassium phosphite were enough to control the disease under high disease pressure (Rebollar-Alviter et al. 2010).

The disease control programs evaluated either as protectant strategy or curative responding to rain events were able to control the disease under weather conditions favoring disease development. Calendar based fungicide applications as well as those responding to rain events take in to consideration the risk of disease development and agree with current recommendation to manage fungicide resistance. Growers have a choice to use the protectant (calendar-based program) or curative strategy under a matted row production system in Ohio and similar strawberry production areas to extend the life of the fungicide by a proper use of fungicide resistance techniques.

As additional factor that contributes to optimize fungicide application for the management of leather rot of strawberry is the distribution of the sensitivity to the tested fungicides. A study was conducted in order to determine the sensitivity of *P. cactorum* to azoxystrobin and pyraclostrobin fungicides among isolates from different parts of the state of Ohio, and other states of the USA, so the risk of resistance development by using these fungicides on strawberry could be determined. The sensitivity of 89 isolates of *P. cactorum* was determined to both fungicides on mycelia and zoospore germination. The results showed that there was a wide distribution of sensitivity to azoxystrobin, indicating a great diversity among the isolates evaluated. Thus, the sensitivity distributions can be used as a baseline sensitivity to monitor shifts in fungicide resistance in *P. cactorum* (Rebollar-Alviter et al. 2007b).

These series of studies showed that both calendar-based and disease risk-based fungicide application can result in a satisfactory disease management. Also a proper combination of protectant and curative approach can extend the life of the fungicide. The results obtained from these experiments are based on growing conditions in the Midwestern US with matted row perennial production; however, it can be also applicable to other type of production systems. For example, in subtropical areas of the central part of Mexico (Michoacan and Guanajuato States), strawberries are grown as an annual crop and season is drastically different from the Midwest; however, rain season coincides with fruit set and first harvest as it is in the Midwestern US. Thus, the same principals for leather rot management can be applied.

## 10. Concluding remarks

In this chapter, we reviewed major components that are associated with fungicide application decision-making process: basic understanding of disease epidemiology; fungicide resistance and its management; fungicide physical mode of action; and use of plant disease risk assessment tools that can integrate these components. We also discussed two case studies where multiple studies are conducted to develop optimal management recommendations. We believe that this chapter demonstrated the complication involved in an optimization of fungicide uses which growers face every day, and presented some of approaches that can be used to investigate this intriguing study subject.

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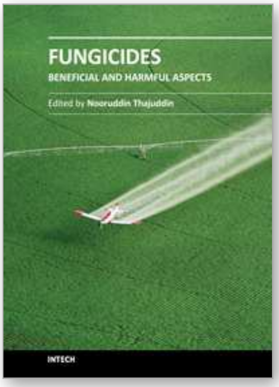


**Fungicides - Beneficial and Harmful Aspects**
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Fungicides are a class of pesticides used for killing or inhibiting the growth of fungus. They are extensively used in pharmaceutical industry, agriculture, in protection of seed during storage and in preventing the growth of fungi that produce toxins. Hence, fungicides production is constantly increasing as a result of their great importance to agriculture. Some fungicides affect humans and beneficial microorganisms including insects, birds and fish thus public concern about their effects is increasing day by day. In order to enrich the knowledge on beneficial and adverse effects of fungicides this book encompasses various aspects of the fungicides including fungicide resistance, mode of action, management fungal pathogens and defense mechanisms, ill effects of fungicides interfering the endocrine system, combined application of various fungicides and the need of GRAS (generally recognized as safe) fungicides. This volume will be useful source of information on fungicides for post graduate students, researchers, agriculturists, environmentalists and decision makers.



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