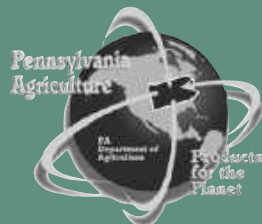# Mushroom Integrated Pest Management

Handbook

The PA IPM Program is a collaboration between the Pennsylvania Department of Agriculture and The Pennsylvania State University aimed at promoting Integrated Pest Management in both agricultural and nonagricultural settings.

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Introduction

In this handbook we have addressed the most important pest organisms with the potential to reduce mushroom yield and quality. The handbook is intended for growers, as well as researchers, as both an educational tool and a reference manual. Recommendations presented here are not intended to bind growers in their decision-making processes. Rather, they should serve as a guide for developing effective Integrated Pest Management (IPM) programs. Each grower should develop specific operating procedures and checklists specifically tailored for individual use. In addition, as technology is always changing, this handbook will be updated periodically.

The handbook is divided into two parts, covering the theory of IPM and the practical aspects of IPM in mushroom growing. The theory section defines IPM and gives it historical perspective. It also explains the concepts of pest management and types of control, and the importance of understanding pest life cycles and biology. The section on IPM in mushroom growing describes how unique features of mushroom crops can be used effectively in IPM, and how the theory of IPM can be applied effectively.

Mushroom growing lends itself naturally to IPM. It is one of the few forms of agriculture in which the crop is grown inside climate-controlled buildings. This offers two advantages not available to most other crops. First, control of the internal environment of the growing room provides an important weapon against many pests. Temperature and humidity manipulations, for instance, are two of many cultural options available in mushroom pest control with IPM. Second, since the crop is grown indoors, pests can be excluded. This control measure is unavailable to farmers of field crops, who have little control over pest invasion. An effective IPM program takes advantage of these particular characteristics of mushroom growing.

Other features of mushroom production make IPM a necessity, not an option. With production measured in pounds per square foot rather than in bushels or tons per acre, mushroom growing is very dense farming. If a pest gets into a room, it can spread rapidly because of the large amount of food available within a relatively small space. In addition, many pests cannot be controlled using chemical pesticides, either because there are no products labeled for mushroom use, or because materials don't even exist for a specific type of pest organism. Increased regulations are driving up the cost of producing new pesticides, making it difficult or impossible for chemical manufacturers to invest in a minor-use crop like mushrooms. Usually, we are forced to rely on pesticides developed for other commodities. An IPM program that excludes pests and takes advantage of the ability to manipulate the growing environment not only is a more effective means of pest control but also allows limited dependency on chemical pesticides.

These features make the IPM approach the most effective and economical means of long-term sustainable pest control. Anyone trying to control pests without IPM eventually will end up at the mercy of those arthropods and mushroom diseases. We hope this manual will help you avoid that fate.

I.

Theory of Integrated Pest Management

A. History, Definitions, and the Economic Threshold

Shelby J. Fleischer

Integrated pest management is the [information-based] selection, integration, and implementation of pest control based on predicted economic, ecological and sociological consequences.

Bottrell, 1979.

Council of Environmental Quality

History, Definitions, and the Economic Threshold

Vernon M. Stern was working for the Westside Alfalfa Pest Control Association in the San Joaquin Valley, California, a big association of growers involving 10,285 acres when it formed in 1945. The association was organized to help decide when to apply insecticides against the alfalfa butterfly.

The alfalfa butterfly was not the most serious pest in alfalfa, but at times it flared up and caused very serious loss. Alfalfa growers had materials like calcium arsenate at their disposal, and they used these materials frequently, but at significant expense and with hard work. The growers formed an association after entomologists showed that a parasitoid controlled the butterfly most of the time, and that growers could make many fewer pesticide applications if they could estimate how well the parasitoid was controlling the butterfly larvae early in the crop growth cycle. The association hired people to do the fieldwork and calculations and to give advice. The Westside Alfalfa Pest Control Association called this "supervised control." The system was successful, and soon the Westley Pest Control Association and the Tracey Pest Control Association formed in other parts of California.

These efforts at supervised control declined rapidly when DDT and other new insecticides came into use. By the late 1940s, over 90 percent of acreage was treated with new materials, calcium arsenate fell into disuse, and the Pest Control Associations disappeared. The new materials worked well for less cost, so Vernon M. Stern went to graduate school with Ken Hagen, the first person in charge of the Westside Association, and Robert van den Bosch, who had also been in charge of the Association for a period of time. They worked with Professor Ray F. Smith, who had initially organized the Pest Control Associations.

It was not long before another insect, the spotted alfalfa aphid, came into the San Joaquin Valley, and by 1955 this aphid was resistant to pesticides. Smith and his students (Stern, van den Bosch, and Hagen) imported an exotic parasitoid and studied native predatory insects. Both the parasitoid and the predators were effective when not destroyed by pesticides. They then found insecticide materials and use patterns that were relatively selective, allowing the natural enemies to coexist with the valuable insecticide tools.

In 1959 Vernon M. Stern with his co-authors Smith, van den Bosch, and Hagen, wrote a paper entitled "The Integrated Control Concept," (Stern et al. 1959) in which they generalized about integrating biological controls and insecticides. To make this work, they discussed monitoring, which requires understanding of sampling and measurement of pest density. They noted how pest populations fluctuate over time. By monitoring density, they argued, intervention with pesticides can be limited. This practice limits chemical applications to those necessary times and places where other tactics are not sufficient.

So when does it become necessary to intervene with pesticides? In many respects, this is an economic decision. It requires relating economics or commercial goals of production to fluctuating pest density. Simply defined, the time to intervene with a pesticide is when the expected gain from using the pesticide equals the costs associated with its use. The pest density at which the gain equals the cost is the Economic Injury Level. Thus, IPM relates pest population dynamics to commercial production goals.

The concept of economic injury level is shown in Figure 1. (Similar figures can be found in Stern's paper, published about 40 years ago, and similar concepts were in use in cotton production almost 75 years ago.) The figure shows that the pest density is changing over time. At low densities, the costs of the damage done by the pests are less than the costs of control, so it does not pay for the manager to add the control. At higher densities, however, it does pay to control. Pest density is dynamic, and managers can make short-term predictions about what the density soon will become. Managers usually want or need to implement controls a short time before the Economic Injury Level is reached. The Economic Threshold is the density at which controls are added. It is set so that, if controls are applied and are effective, the Economic Injury Level is not reached.

This "Integrated Control" concept, published in 1959, was quickly expanded to include all methods of control. Thus, the Integrated Pest Management concept was born at least 40 years ago. The concept was not born solely in California; similar developments were occurring in Arkansas for cotton crops and in Canada for apples. The concept arose from a philosophy for which the objective is to manage a pest population below economically damaging levels, and in a way that is practical for growers, by integrating multiple control options (see Perkins 1982 for a full historical perspective). IPM always has emphasized integration of control tactics, including pesticides, and monitoring to help determine time and location for pesticide application.

Figure 1. Graph of economic injury level.
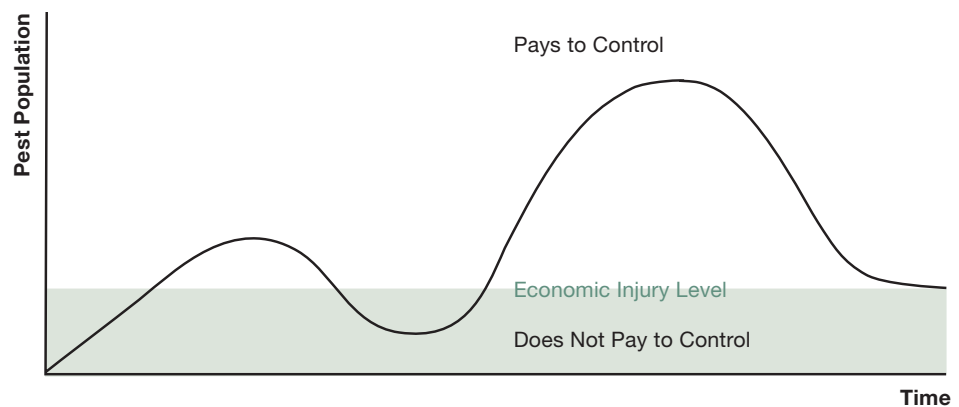
Problems With Pesticide Overuse

Over time, well-documented problems with sole or over-reliance on pesticides were discovered, and they still are being discovered. Use patterns, rates, timing, and other aspects of pesticide application are designed to minimize these problems. Pesticides are an important part of IPM, but not the only part, and they require a good understanding to be used well. To ensure safety and conduct business legally, it is essential to follow the label and to realize that changes to labels occur frequently. Information for pesticide use is printed on the label, which is a legal document, and must remain with the pesticide container. Over-reliance on pesticides has been linked to problems, including:

Resistance
A change in the genetics of the pest population that impairs control in the field.

Depletion of natural controls
Mortality of predators or parasitoids, which results in pests reaching even higher densities (called resurgence) or species that were not previously pests reaching pest status (called secondary outbreak).

Biomagnification
A build-up of the pesticide in fatty tissue, followed by an increase in the concentration of the pesticide in organisms higher on the food chain, including humans.

Environmental contamination
Unacceptable levels of the pesticide in groundwater, or in parts of the environment where pesticides were never meant to be.

Species displacement
A change in the biodiversity of an area caused by the effect of pesticides on species populations.

Endocrine disruption
Pesticide (and other) molecules acting upon the hormonal system of animals and humans, affecting their development and immunological processes.

Human health danger
Direct or chronic toxicity to applicators or manufacturers; or to consumers caused by unacceptable residues in food.

Today, there are many definitions of Integrated Pest Management. They all recognize that many factors influence pest dynamics, the way these dynamics relate to production agriculture, and the need to integrate multiple control strategies to manage pests over a long time frame. IPM has foundations in ecology—an understanding of the relationships of the pest and beneficial organisms within the biotic and abiotic environment, and an understanding of the distribution and abundance of these organisms. IPM emphasizes creating conditions to preclude an organism from reaching pest status, correctly diagnosing and monitoring pest pressure, and allowing natural mortality factors to work as well as possible. IPM is a philosophy, a way of thinking, an attitude, which is adapted in practice to meet economic realities of commercial production and modified as those realities or tools available for management change. A slightly modified definition of IPM from the Council of Environmental Quality is ". . . the [information-based] selection, integration, and implementation of pest control based on predicted economic, ecological and sociological consequences" (Botrell 1979).

An IPM Philosophy in Mushroom Production

IPM in mushroom production got its start when sciarid fly populations began to explode in the late seventies as the result of environmental changes brought on by the availability of air conditioning. Before air conditioning, mushrooms were produced only in the cool season. When crops were most susceptible to infestation, it was usually too cold outside for wild populations to be mobile, thus they were not able to enter growing rooms. Also, the summer was a break in the growing cycle, and thus also a break in the life cycle of sciarid flies within mushroom houses.

With the advent of air conditioning, there was no longer a break in the growing cycle, and sciarid flies were able to breed uncontrollably. Despite the use of chemical pesticides, the flies were winning the battle. By the summer of 1978, fly populations in Chester and Berks Counties in Pennsylvania were causing severe crop loss.

The Pennsylvania State University began an interdepartmental Integrated Pest Management program for the mushroom industry in early 1979. The goal of the program was to reduce pest populations in an ecological way to economically tolerable levels. The program was to study four major mushroom pests and diseases: the sciarid fly, *Lycoriella mali* (Fitch); the phorid fly, *Megaselia halterata* (Wood); *Verticillium* or dry bubble, *Verticillium malthousei*; and bacterial blotch, *Pseudomonas tolaasi*. The most important components of the program were monitoring and identifying pests and diseases. Mushroom pest adults as well as larvae and eggs were monitored and identified, and mushroom beds were sampled for diseases so their life cycles could be studied.

We easily can recognize an IPM philosophy in past and current management of pests in mushroom production. Management tactics are related to the biology and ecology of pest species and the relationship of the pests to yield, quality, or marketability of the crop. There are a variety of tools in mushroom production that influence pest density and dynamics. These are not mutually exclusive, and are best integrated so that one tactic helps another. Many specific strategies with specific pests are discussed in the following chapters, but it is worth pausing to consider general terms that classify control strategies and their relevance to mushroom production:

Exclusion
Techniques that help prevent the pest from reaching sites where it can create damage, such as sealing walls and cracks, to prevent entry of flies. Air must be filtered before it enters the rooms. Any personnel or equipment entering a room must be clean and/or sanitized.

Delaying access
Techniques that slow the rate at which a pest reaches sites where it can create damage. Examples include maintaining sanitation in the premises around mushroom growing houses and keeping grass cut and trees trimmed.

Cultural control
Growing techniques that make the environment less supportive of pests and more supportive of beneficial organisms. Composting is an excellent cultural IPM technique that strongly influences fungal competitors and pathogens. Instigation of shorter crop cycles is another IPM tool that strongly influences pest population dynamics. Also important is maintaining an environment, including proper temperature and relative humidity, that favors mushroom growth over its competitors'.

Sanitation
Here's where mushroom growers can excel compared to many other agricultural production systems. The controlled environment required for mushroom production allows for use of steam-pasteurization at the beginning and end of the crop, and sanitation of the growing rooms and equipment.

Biological control
Influencing the density or activity of beneficial organisms, either through cultural management or inundative release of additional beneficials into the environment. Composting techniques influence biological control of fungi. Purposeful release of Pteromalid parasitoids on the composting wharf, or entomopathogenic nematodes, are examples of inundative release of beneficials used in mushroom production.

Chemical control
Introducing chemicals to kill pests. The types of chemical tools available are changing rapidly, and mushroom growers have kept up with this change. Compare, for example, the types of materials listed in Duffy 1981 with Fleischer and Keil 1994. The 1970s relied on broad-spectrum materials that had activity against a wide range of insects; the 1990s relied more on insect growth regulators that have much greater selectivity and are more precise in what they target. This trend of greater selectivity can be expected to continue. In an IPM program, these chemical tools are used in a way that is as compatible as possible with the other tools listed above, as well as with pesticide resistance management, which is discussed later in this chapter.

Biorational materials

Synthesized or extracted compounds that are applied to manage pest densities, which often have much greater selectivity upon target pests. Examples used in mushroom production include insect growth regulators, botanical extracts, and microbial metabolites.

Clearly, mushroom growers can and do integrate multiple control strategies, as in an IPM program. Further, there is the issue of relating decisions to pest population dynamics: the Economic Injury Level or Economic Threshold. The level of pest density that can be tolerated is both a management and subjective decision. Not every farm is the same. Both the gain achieved by the use of the material and the market price of the mushrooms will vary among farms and through the season on a given farm. Economic goals also vary: some farms may emphasize quality factors for select markets, others may emphasize volume.

In addition, other biological factors should influence management decisions about the tolerable levels of a pest. For example, if sciarid flies are aiding transmission of a pathogen or mites, then the tolerable pest density of flies should drop dramatically. The tolerable pest density is also different at different times in the crop growth cycle. This tolerable pest density is best developed through experience and in consultation with others who have had growing experience.

In an IPM philosophy, growers do not strive to remove every individual pest at every moment. Rather, management involves monitoring pest pressure and using that information to influence management. In mushrooms, monitoring includes fly monitors and record sheets (Figure 2), nuisance fly monitors on the composting wharf, and routine inspection of beds for diseases. Tem-

peratures are monitored in both Phase I and II. Though this is not a direct measure of populations, it is a good indicator of what is happening microbially; if the compost is cold near the center of the pile—for example, 120°F (49°C)—it is an indication that there are anaerobic organisms producing the wrong type of compounds. This is not a direct measure, but it is very important to the quality of the compost and reminds the growers that the compost is alive, something that usually gets very little attention.

Rules of thumb provide economic injury levels for some pests. For example, fly pressure may be low enough during the winter to not require insecticides. An economic injury level of adult sciarid counts per day, as determined on the Pennsylvania Mushroom Fly Monitor, is shown in Figure 3. In this example, there is virtually no tolerance for flies before, and 3 days after, spawning. After spawning, the threshold rises to ten flies per strip per day. The threshold rises again slightly soon after casing and more dramatically at pinning. The idea is that flies arriving early will cause greater damage and are a sign of much greater problems that will occur before the crop is complete, but flies arriving later will have less opportunity to cause damage because they have less time to complete another life cycle. This specific threshold may not be the best for your facility, as the cropping cycle and other factors may not be exactly the same, but it does demonstrate that thresholds can influence management and suggests that thresholds can be adapted to your farm.

Monitoring is essential for defining when and where to invest pest management inputs. The first step in monitoring is identifying the pest and diagnosing the problem. Monitoring also is essential for *evaluation* and *follow-up*. It

determines whether or not a pesticide or other management strategy is working and calls attention to times when strategies are not working as expected. Sometimes pest pressure increases after a pesticide is applied. Perhaps new immigrants arrived, or they arrived more quickly than anticipated, or a stage of the pest that was not susceptible to the pesticide developed into a stage that is now a problem. Perhaps the pest population is developing resistance to the pesticide currently in use. Clearly, monitoring is an essential part of IPM.

It is clear that the philosophy of IPM is compatible with mushroom production. The *Penn State Handbook for Commercial Mushroom Growers* (Wuest 1992) is filled with valuable information about identification, diagnosis, cultural controls, monitoring, and management. A basic premise is that no single control method will be successful over time. IPM strives to integrate control tactics, which essentially are different types of technologies. IPM will use cultural and biological tactics to the best degree possible and then include pesticides as needed. Control technologies discussed in this publication include Diagnosis and Monitoring, Exclusion (Chapter II.A.1), Cultural Controls (Chapter II.A.2), Biological Controls (Chapter II.A.3), and Chemical Controls (Chapter II.A.4).

Technologies change over time. What mushroom growers may not realize is that they can be among the best at adapting to these changes. Changes in technology are true for cultural technologies as well as for biological and pesticide technologies. Consider the change in growing technology, varieties, and cropping cycles over the last 20 years. Because the technologies keep changing, the IPM program also must adapt, change, and improve. It is clear that the IPM philosophy of integrating

Figure 2. Pennsylvania mushroom fly monitor record sheet.
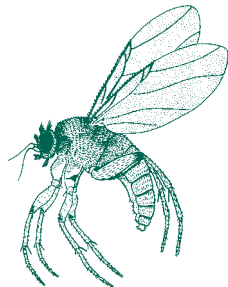
Pennsylvania Mushroom Fly Monitor Records

Block Number: **Room Number:**

Day	Date	Number and Name of Flies	Day	Date	Number and Name of Flies
-6			+8		
-5			+9		
-4			+10		
-3			+11		
-2			+12		
-1			+13		
Spawning			+14		
+1			+15		
+2			+16		
+3			+17		
+4			+18		
+5			+19		
+6			+20		
+7			+21		

Cecid Fly   Phorid Fly   Sciarid Fly

Instructions for use: Record daily fly counts from the monitor for days -6 thru +21 from spawning. Note whether the flies are cecid (=C), phorid (=P), or sciarid (=S).

Note: A 10x hand lens will be helpful in insect identification.

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Figure 3. Pennsylvania mushroom fly monitor action levels. Adult sciarid fly counts determine the need for insecticide applications in a growing room.
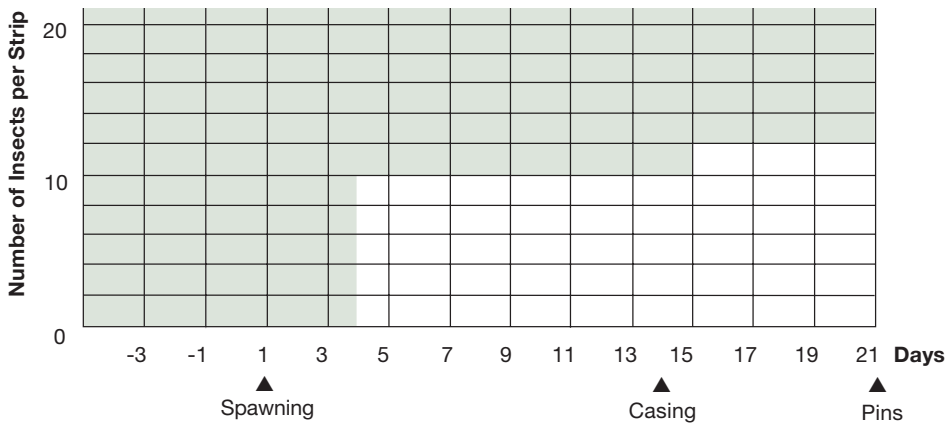
multiple management tactics and allowing pest density dynamics to influence management is well embedded in the modern mushroom farm.

In subsequent chapters, note how often a range of management options are discussed and how these options require an understanding of the life cycle of the pest and an understanding of how the pest interacts with the biotic and abiotic environment (in other words, the ecology of the organism). In the future, new technological options will become available, and IPM is a philosophy that can integrate and prioritize these options. Fundamentally, an IPM program identifies and monitors the pest, takes advantage of the options that manage the pest through cultural means, and adds pesticides when needed. The new pesticides will bring improved safety and environmental profiles. To be preserved, they should be implemented in conjunction with a pesticide resistance management program.

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B. Pesticides and Resistance in IPM

Shelby J. Fleischer

Changes in technology are easy to see. What may not be as obvious is that the pests themselves keep changing. Perhaps the most important change is the development of resistance. In many agricultural systems, resistance has been the single most important factor causing the decline of a pest management strategy.

Resistance is a genetic change, occurring in response to selection by toxicants, that may impair control in the field. Pests can withstand toxins to some degree, often in relation to the dose to which they are subjected. There is variation in this ability to detoxify; that is, some individuals can detoxify more easily than others. Pesticides are one type of toxin, and when they are applied, individuals in a population are killed. If individuals with improved ways to detoxify exist, selection for those individuals will inadvertently occur. They will survive and reproduce more easily than other indiviuals in an environment that includes the pesticide. This process is known as selection for resistant individuals by the toxicant. Continued selection will result in a resistant population. This is evolution in action, and it is the same process that results in strains of human pathogens becoming resistant to antibiotics.

Evolution of resistant pest populations is a common fact of agriculture today. Over 500 pest insect species have evolved resistance to at least one pesticide during the last 40 years (Georghiou and Taylor 1986). The increase in numbers of resistant species has been exponential for these last 40 years. More recently, the increase in resistant populations of pathogens and weeds are beginning to follow the same curve. It should come as no surprise, therefore, that resistance in mushroom pests is now well documented. Examples include sciarid flies, which are resistant to pyrethroids (Keil and Bartlett 1996); house flies and stable flies on the composting wharf, which are resistant to many classes of insecticides; and verticillium, which is resistant to benomyl.

Resistance must be evaluated with respect to the natural variation among individuals and populations in their abilities to detoxify a pesticide. It can be a matter of opinion as to when to label a population as resistant, and when it is just displaying natural variation. The World Health Organization has set a standard of 10; that is, when a population requires 10 times the amount of pesticide to kill 50 percent of a test population compared to a reference susceptible population, it is classified as resistant. Also, it is very common for populations to exhibit different abilities to withstand pesticides in different geographic areas. Thus, a pest may be resistant in only certain, often small, geographic areas. With this in mind, it also is possible to recognize limited resistance of sciarids to diflubenzuron.

Resistance Management

The realistic potential for resistance is a predictable, evolutionary consequence of pesticide use (and other management tactics as well). Therefore, resistance management is now considered and must be a part of integrated pest management. As a new management tactic such as a new chemical is deployed, it should be used in a manner that is designed to prevent or slow the development of resistance. This is becoming especially important as we move to the use of newer, selective materials. The goals of resistance management are to avoid resistance, slow the rate of resistance development, and cause resistant populations to revert to more susceptible populations.

To best understand resistance management, it is helpful to understand the details of the evolutionary process that results in resistance. When measuring something about an *individual*, such as its ability to withstand a pesticide, you are describing its phenotype. When measuring phenotypes for a *population* of individuals, the phenotype of that *population* can be described (for example, you may observe that 20 percent of a population withstands a specific dose of a specific pesticide). With resistance, we observe reduced rates of mortality (lower efficacy) when a pesticide is applied.

Lower efficacy can be due to many causes. In fact, in most cases in agricultural settings, lower efficacy is caused by application, timing, or something that is not related to resistance. However, when lower efficacy is caused by a change in the proportion of the pest population that carries a heritable genetic component such as DNA, then lower efficacy is due to resistance.

Mutations cause the variation of DNA among individuals. Mutations are rare (perhaps one in a million at a given site), but they are present. For example, if mutations occur at a rate of one in a million at a given site on a long strand of DNA, and there are 100 million such sites in the DNA of a human, then there are about 100 mutations occurring in each human. In DNA, which codes for protein, mutations result in different versions of the same protein. Most mutations have either no effect or are harmful. Some, however, produce beneficial results; some proteins provide individuals with improved abilities to survive and/or reproduce.

Principles of Resistance Management

When a pesticide with a new mode of action is introduced into commercial use and gains acceptance, it can be assumed that it is effective. At that point, it kills the target pest, and resistance is not a problem. What has been learned from many experiences with pests that have evolved resistance is that alleles (segments of DNA that code for protein) that confer resistance are either not present or are present at very low frequencies when the new material first is used. These low frequencies are often lower than can be measured economically. For the purposes of this exercise, assume that resistant alleles are present in less than one in 100,000 individuals.

When this same pesticide is observed by a grower to be not as effective as it used to be, and assuming that everything else is the same, then resistance is probably occurring. By that time, enough individuals are carrying resistant alleles to make it visible to a grower. For this to happen, the resistant individuals would have to occur reasonably frequently; for

example, one in 1,000 individuals now would be surviving the pesticide treatment. That represents a 100-fold increase in the frequency of resistant individuals! The key to effective resistance management is to start a resistance management program early. Do not wait until field failures become obvious; by that time, a dramatic increase in the frequency of resistant alleles has already occurred. The best time to design a resistance management program is before a new product is ever used.

Crop protection companies are anticipating the evolution of resistance to their new materials and are providing resistance management programs as part of the initial introduction of a new material. In some cases, companies are monitoring for resistant alleles at the time of introduction, with sensitivity that would detect the very low levels expected in the early stages of resistance development. Pesticide Resistance Management (PRM) is becoming a part of IPM.

Factors Affecting Resistance Management

The development and rate of resistance are affected by genetic factors, biological and ecological factors, and operational factors-activities performed within and surrounding a production facility (Georghiou and Taylor 1986).

Genetic factors refer to the genetics of the pest itself. Does the capacity for resistance exist? Do some individuals in the population have alleles that code for proteins that confer resistance? Do some detoxification proteins of some individuals work faster? Do some have thicker cuticles that slow the rate of pesticide entry? Genetic factors vary—pests do have mutations—and it is possible, although rare, that a new mutation will confer resistance. For purposes of long-term resistance management, it should be assumed that, at some level, resistant alleles are present. For the purpose of the following illustration, we will indicate resistant alleles, pieces of DNA that code for proteins that confer resistance, with a capital "R." Susceptible alleles will be indicated with a capital "S." Most insect pests have two copies of each allele, so they may be indicated by "RR," "RS," or "SS."

So what is the frequency of resistant alleles—what is the percent of the population that displays resistance? The higher the frequency, the higher the rate of development of resistance. The R allele is mixing every time an insect mates. If RR individuals mate with SS individuals, offspring will be RS, helping to dilute the R allele. Possible combinations include:

RR with RR to give RR

RR with RS to give RR and RS

RR with SS to give RS

RS with RS to give RR, RS, and SS

SS with RS to give SS and RS

SS with SS to give SS

When a pesticide is introduced into effective commercial use, pest individuals are almost entirely of the SS type. As resistance develops, some RS become present (from one in 100 to one in 10,000), and there are many, many fewer RR (from one in 10,000 to one in 100,000,000). Even if R alleles are present, it is desirable to keep many SS individuals nearby and mating, slowing resistance development. So if the population can be swamped with susceptible individuals, resistance can be slowed. This is important, because most of the population (say, from outside the mushroom house) consists of susceptible individuals (SS) during early stages of resistance. In the early stages of resistance, the very rare RR individual might have a greater chance of drowning or desiccating—or dying from any number of causes—than mating. After a pesticide application, some individuals with R alleles may survive, but some with S alleles might also (they may have been in a protected growth stage, like the egg stage, and may not have been affected). As long as we can keep the frequency of R low, we have an effective resistance management program.

With few important exceptions, the R allele probably is mildly deleterious. For example, it may mildly reduce fecundity, at least initially, in the absence of the pesticide. The initial R frequency is held in check by a balance between mutation and selection, although exceptions are important because they lead to stable resistance. However, in the presence of the pesticide, the R allele confers an advantage to individuals that contain it. Over time, the R allele is balanced with other alleles so that it may no longer be deleterious.

Biological and ecological factors refer to the biology and ecology of the pest. Reviews of the many pest species that have evolved resistance have shown some clear patterns. One important example is generation time. Pests that quickly speed through one generation after another have a much greater potential of evolving resistance in response to selection by pesticides than pests with slower generation times. Similarly, pests that have a high reproductive potential—each female generating many offspring that survive long enough to reproduce—evolve resistance quicker. Immigration traits also are important, but tend to work in the opposite direction. Pest species that have higher rates of immigration tend to have slower rates of resistance, because the constant flow of S alleles into the population serves as a resistance management tool. Those species with low rates of immigration have greater chances of RS or RR individuals mating with each other, which rapidly increases resistance.

The host range of the pest also has shown a trend. Pests that have populations spread out among many hosts (polyphagy) tend towards lower rates of resistance than those that specialize on one host. This is because there is a tendency for patches of pest populations to exist on untreated areas, or refuges, and susceptible individuals existing in untreated refuges serve to maintain S alleles. Pests that have many matings (polygamy) also tend toward lower rates of resistance, because there is less chance for RR individuals to occur.

Operational factors are under the grower's control. These include the timing and dose of a pesticide, choice of materials, decisions about tank mixing, and decisions about alternating products. Dosage, or application rate, often determines if an individual is susceptible or resistant. At some very high dose level, every individual will be killed, and at some low dose level all individuals will survive, regardless of whether they carry R or S alleles. Measurement of the relationship between dose and mortality is a dose-mortality curve. The dose-mortality curve shows the proportion of the population killed on the y-axis against the dose on the x-axis. By changing the way the numbers are represented, we can straighten out the dose-mortality curve into a line. For a single population, the curve looks like this:

Figure 4. Dose-mortality curve.
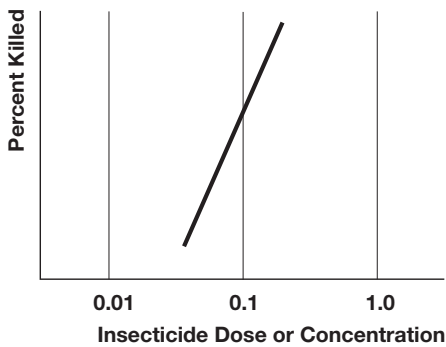
When resistance occurs, the curve changes. The measured points don't match the line as well, suggesting greater variability in the relationship; or the curve shifts to the right or gets more shallow, predicting that mortality is lower at the same dose, like this:

Figure 5. Dose-mortality curve with insecticide resistance.
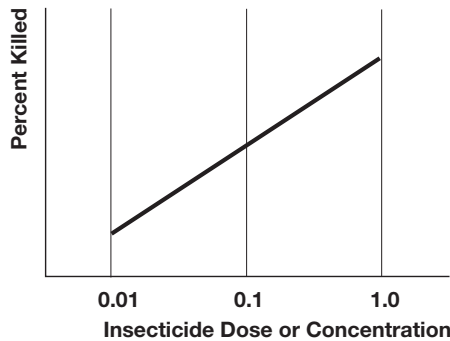
Thus, one way of monitoring for resistance is to plot the dose-mortality relationship from different points in time, or different geographic areas, or different populations. Different populations of SS, RS, or RR individuals will result in different lines, like this:

Figure 6. Dose-mortality curves for three populations.
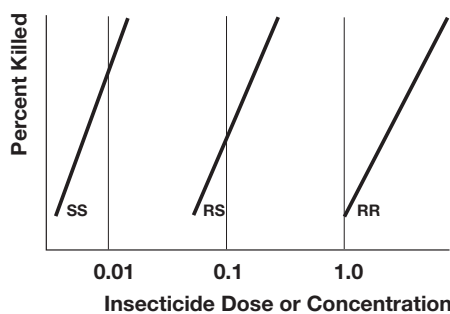
The dosage applied determines selection for resistance. If the dosage applied is high enough, SS, RS, and RR individuals are killed, and no selection is taking place—an event that rarely happens in the real world. Even when it is possible to apply such a high dose, the pesticide will decay over time, and new pest individuals that arrive (either from immigration or development from another life stage such as an egg) are then experiencing a reduced dose.

When a dose kills some individuals but allows others to survive, it is called a selective or discriminating dose. A dose that is able to separate genotypes (RR from RS, or RS from SS), is a discriminating dose. This might occur when a pesticide is applied to a mixed population, or it might occur after a pesticide has been applied and is degrading into a lower concentration. Figure 7 shows a selective dose, where SS and RS individuals are killed but RR individuals survive.

Figure 7. Dose-mortality curves for three populations, including selective dose.
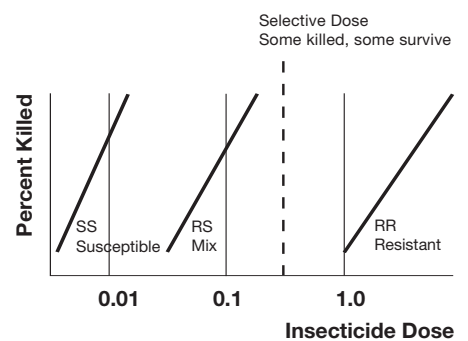
In the example shown in Figure 7, the RS individuals are killed, but at a lower dose. In the following example (Figure 8), selection might kill only SS individuals. This (Figure 8) is the worst-case scenario for resistance management, because there typically are more RS than RR individuals. When the selection is allowing RS individuals to survive, the R allele will increase more rapidly because there are more RS individuals.

Figure 8. Dose-mortality curves for three populations, including less-effective selective dose.
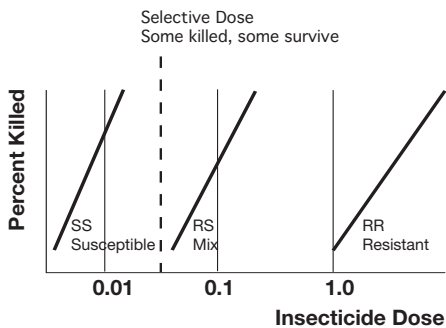
These examples also illustrate that the dominance of the R allele—whether resistance is expressed in RS individuals—depends on the dose. When the dose is selecting for RR individuals and killing RS individuals, the R allele is recessive or not being expressed when it is combined with the S allele. But when the dose is selecting for both RR and RS individuals, the R allele is dominant. The effective or functional dominance varies under field conditions and depends on dose.

As can now be seen, population dynamics and population genetics interact. With resistance management, the population of alleles (R and S) and the population of individuals (the density of individuals of each type) must be considered. For example, there can be an unstable equilibrium, where R is selected for but not maintained at high levels. This can occur within a large population where RR exists at a low level. A discriminating dose selects for the RR individuals, but there are few of them. If high rates of immigration and mating of SS individuals follow, most of the offspring will be SS and RS, although some RR will occur. Population density, population genetics, and resistance are fluctuating over time, and resistance management, as stated earlier, is striving to avoid resistance, slow the rate of resistance, or cause resistant populations to revert to susceptible populations.

Strategies and Tactics of Pesticide Resistance Management

Applying the aforementioned theory to different strategies can help manage resistance. These strategies have been classified as saturation, multiple attack, or moderation, and have been tested with simulation models and limited field experiments in various agricultural systems.

Saturation is an effort to prevent selection by making sure even resistant individuals are killed, typically with a high dose, and sometimes adding synergists to block detoxification. It has been dubbed the "high dose, high risk" strategy, and it works well if all pests are killed every time. To work, it needs to be started while the initial R frequency is very low, and not after some concern about efficacy is occurring. When pests re-invade, the saturation strategy works best when the immigrants are susceptible individuals that mate randomly with the resistant ones, which is difficult to achieve in an environment of high dosage. The saturation model also works best on pests with a low reproductive potential. Also, this strategy has high risk, because once it fails (when the dose is no longer killing all individuals but allowing some RR, or even worse, some RS, to survive), it will continue to fail quickly if it is not changed. It may be difficult on some mushroom farms to deliver and maintain a sufficiently high dose at all locations that need to be targeted. When using the saturation model, it is important to remember other concerns surrounding use of large amounts of pesticides.

The multiple attack strategy takes aim at different modes of action with rotations or tank-mixes of different materials. Rotations involve switching

materials for different applications and require a good choice of material in the rotation. Ideally, any resistant individuals surviving the first pesticide application are killed with the second material. As in saturation methods, rotation works best when started very early, well before field failures are noted. This is because the few resistant survivors would have less chance of mating and producing offspring that also mate when their population is very low. When populations are very low, lots of natural mortality (drowning, desiccating, disease, etc.) can keep them low. Issues about whether the R allele exists, and whether cross-resistance exists, should influence choice of material. To work over a long time frame, rotation assumes that the frequency of R to each material declines while it is not being used. This may occur when the next pest immigration is composed mostly of susceptibles (see below). Rotation also assumes no cross-resistance.

Tank-mixes also combine materials, but at the same time. They are sometimes used to help ensure efficacy. Some argue that tank-mixes also can be a resistance management tactic. Tank-mixes with materials of distinctly different modes of action may help ensure that the second material kills the rare individual that is resistant to one material. However, if a farm starts to tank-mix because a material is not working as well as before, it may be too late—the resistant individual may not be so rare anymore. Tank-mixes also add expense, and if problems arise, they are harder to diagnose. If the different materials do not degrade in the same way, the pests are not really exposed to both materials at some time after the applications. In simulation models, tank-mixes work best when started early, while the R frequency for any material involved in the strategy is low, and when the

frequency of individuals resistant to both materials is exceedingly rare. The assumptions are that all individuals are susceptible to one or both materials, the materials decay at approximately equal rates, and as in rotation, there is no cross-resistance.

Cross-resistance refers to resistance that developed against one material also conferring resistance to another material. Cross-resistance has been fairly common for some insects and some classes of modes of action. Cross-resistance has occurred from one pyrethroid to another pyrethroid, from the old organochlorines to the newer pyrethroids, and from organophosphates to carbamates. This is because there are some similarities in the modes of action of these materials at the molecular level. To avoid cross-resistance, choose materials with distinctly different modes of action. With insecticides, current options include insect growth regulators, pyrethroids, entomopathogenic nematodes, and protein crystals from *Bacillus thuringiensis*. There are new materials with yet other modes of action in the pipeline as well, including microbial metabolites that affect GABA receptors, nicotinoid materials, botanicals, and newer insect growth regulators that target different parts of insect development. Investment in research will help develop these materials for mushroom production. All of these have very different modes of action—some are better classified as biological control materials, and their integrated use helps make clear how pesticide resistance management is consistent with the philosophy underlying IPM.

Moderation strives to maintain susceptible individuals in the population using all IPM tactics (cultural, exclusion, mechanical, biological, etc.). Moderation attempts to *preserve susceptibles* in the environment and allow mating of these SS individuals with those carrying the R allele. The goal is to keep the R allele swamped with S alleles. Growers should use application timing to try to preserve susceptibles early in the evolution of resistance, so that not every pest individual is targeted at every moment. Monitoring and timing applications help preserve susceptibles. Creation of refuges (refugia)—areas that are not sprayed—also preserves susceptibles. Refugia can be in the mushroom house itself or in surrounding habitat if they contribute to mating.

In many studies, the decay rate of pesticides has strongly influenced the rate of resistance, and fast-decaying materials are associated with the moderation management strategy. Materials that decay quickly initially have a high (and hopefully non-selective) dose, killing all genotypes. Then the fast-decaying materials are gone. There is little time during which the dose is selective. Materials that decay slowly go through a longer time with a selective dose. In general, slow-decaying materials—those often credited with "residual activity"—favor the development of resistance. They can exhibit selective activity over longer times and make it harder for immigrating SS individuals to survive and mate with the rare RR individuals that are surviving. Choosing materials with a fast decay rate has worked as a resistance management tactic for house flies.

In most field examples to date, pesticide resistance management programs have required preservation and mating with susceptibles. Choice of short-residual materials has worked in models and in practice. Limiting application to specific times of season or generations of the pest, or leaving refuges of untreated areas with immigration of susceptibles from those areas, have been useful and may require coordinated activities of neighboring growers. The most important factors in simulation models suggest that resistance is most influenced by the reproductive potential of the pest; also, that resistance is best slowed by immigration of susceptibles and reduction of selection pressure by making applications only when needed, carefully choosing the dosage, and using shorter-residual materials.

One take-home message is that mixtures, rotations, and saturation all require conditions not well met in the field; reducing pesticide use (via IPM) has proven more productive than optimizing pesticide combinations and spatial deployments. Pesticide resistance management has relied on knowing pest biology and ecology, understanding evolution, and integrating management tactics. Technologies available for pest management are changing constantly to keep up with changing conditions for growing and marketing the crop and with changes in the pests themselves. A resistant pest population is a change in the pest population. Clearly, pesticide resistance management has a philosophical basis and is part of IPM.

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II.

Integrated Pest Management in Mushroom Production

A. Specific Control Techniques

1. Exclusion

Phillip S. Coles

Exclusion prevents the entrance of pest organisms into new rooms and their escape from older ones. The latter should not be underestimated. Pest populations usually are high in older rooms, and they threaten infestation of younger crops if they are not contained. Since mushrooms are grown inside environmentally controlled rooms, our industry is in a unique position in agriculture: we are able to control pest movement into and out of growing rooms. This must be exploited fully in any mushroom IPM program. Once a room is pasteurized successfully, pests will have to enter in order to become a problem. If exclusion were completely successful, there would be no need for *any* other form of pest control for most diseases. (Some organisms such as the bacteria that cause blotch are not destroyed by pasteurization and must be controlled through other methods.) This is especially true in the winter months when pests should be virtually nonexistent.

Exclusion, like monitoring, is often discarded when another "magic bullet" pesticide comes onto the market. The pesticide will give good control for a time, then resistance (See Section I.B) will begin to occur, reducing the pesticide's effectiveness, or worse, rendering the material useless. Exclusion limits the number of pests exposed to a given pesticide, thereby reducing resistance.

There are several ways to accomplish exclusion: the integrity of the building must be maintained; openings must be secured (doors, fans for boiler or electric rooms, etc.); air entering rooms must be filtered; and the movement of people and equipment must be restricted.

Construction of new growing rooms must permit easy sealing of the building and provide easy maintenance of that seal. All areas should have easy access. Any areas not exposed for easy inspection can allow openings to form undetected. Moldings along rooflines, for example, can hide cracks between the wall and roof. Sometimes air handling transitions or ducts are not installed tight against the ceiling. The space between the duct and ceiling can be so small that it is impossible to seal the area where the wall and ceiling join over the duct. Remember, the extreme environmental conditions produced during a normal mushroom crop, particularly during pasteurization, can be very stressful on a building. Cracks can develop that were not there during inspection prior to the crop.

Building materials also are important. Because it is organic and porous, wood can be a good hiding place for pathogenic organisms. Porous cinder blocks and concrete also provide refuges for organisms, particularly in the floor, where it is nearly impossible to develop high temperatures. Consider inorganic,

smooth, dense construction materials whenever possible. Plastic and aluminum are good choices, though cost often precludes their use. Inorganic insulation is a must. Sawdust, for instance, can become a breeding ground for pest organisms. Cost of materials must be weighed against potential benefits.

Don't overlook the obvious entry points in any building, such as drain holes (Figure 9) or the webbing in blockwork. Unless the top of the wall is capped, there are thousands of passageways within the wall through which flies can pass.

In an existing facility, mortar and caulk are inexpensive alternatives to chemical pesticides or crop loss. When a growing room is empty, inspect for cracks and any other damage that may have occurred during the crop. Buildings expand and contract from the changes in temperature during the crop. High humidity causes wood to swell. Where disimilar materials come together, such as wooden doorjambs against block walls, the different expansion rates of the materials cause cracks to develop between them. All of these areas must be inspected, sealed as needed (Figure 10), and marked off on a checklist. Turn off the lights inside the room and look for light penetration from outside. If a growing room has a spring roof, this area must be checked. Ceilings are especially susceptible to damage, particularly if the temperatures during pasteurization are allowed to get too high. High temperatures can damage insulation; sprayed-on polyurethane can buckle and crack. Nailed insulation sheets can buckle, pulling the nail heads through the insulation and leaving access holes through which pests can enter. Turn on the lights inside the room when inspecting a loft area and watch for light penetration. Pasteuriza-

Figure 9. Drain plugs must be sealed to keep out flies and especially rodents.

Figure 10. Rooms can be sealed with urethane insulation.

tion at the end of a crop is also a good time to check lofts, since steam will escape through openings in the ceiling. Mark these openings and have them repaired. This not only will exclude pest organisms, but will reduce energy costs as well.

Limiting and sealing access doorways is of particular importance. Only one or two doors in a plant or any mushroom building should be used as entrances. All other doors should be sealed. Doorways used for entrance and exit must be sealed around the edges, and there should be a threshold at the bottom to seal the door when closed. Seal these doors with weather-stripping or strips of filter material (Figure 11). Spray the sealed edges with oil or adhesive as an additional barrier against pest entry. A step mat with a sanitizer should be placed at the entrance to sanitize shoes. Clean the mat regularly or it could become a source of infestation. It is better to not have any mat than to use a dirty one. Entrance doors into the growing rooms should be treated the same way as the entrance to the hallway or the plant itself. If there is more than one door into the growing room, one of them can serve as the entrance and the others can be sealed completely. If doors must be kept open for ventilation during Phase II, they should be covered with fly netting or filter material.

Obviously, filter media must be impervious to fly penetration. What may look impervious to us may not be to a fly. Flies can get through much smaller holes than their body size suggests. Filter media offer an additional problem. The structure of most filter media makes it ideal for collecting dust particles, but also for active pests, such as flies, to work their way through it. When a fly comes in contact with filter material, it sees a mass of hair-like

Figure 11. Filter material used to seal doors.

fibers. The fly will move the fibers back and forth and work its way through material that looks impenetrable. Only by testing the filter material or fly netting can a grower be confident flies cannot get through it.

Choosing Filter and Fly Netting

Testing can be accomplished in a variety of ways. First, inspect the material. Holes large enough to permit the passage of flies may be obvious. Netting or filter media may be manufactured inconsistently, allowing some of the openings to be larger than others, or a filter may have thin areas or "windows" insects can pass through. The material may not be strong enough: i.e., people working near netted openings may damage it too easily.

Second, test the filter or netting for fly penetration. One method to accomplish this is to wrap the netting or filter media in question around a wire frame and place a known number of flies inside. Seal the material with a twist tie or string and place it outside (where the flies can't have access to new growing rooms) or inside an old room that has flies, and determine if they escape. Conversely, you could place something the flies want inside the material and determine if they can penetrate it. Try placing fresh spawned compost or a fly light equipped with flypaper inside a box to capture invaders. Use an open topped box with sealed seams and place freshly spawned compost in the bottom of the box. Place flypaper on the top of the compost, sticky side up. Or, attach a fly light with flypaper to the bottom of the box. Cover the open side of the box with the material and seal it with duct tape. Make sure there are no openings in the box or at the seals where flies could get through. If a fly light is used, an opening must be made for the power cord. Be certain this is sealed. Put the box in an old, fly-infested room. If the test material is impervious to fly entry, no flies should be found on the paper.

There are other considerations for choosing filter material instead of netting for a particular application. With netting, excluding flies is enough, but filters are expected to remove spores and dust particles. Spores of concern in mushroom cultivation are from two to ten microns in diameter. (A micron is one millionth of a meter, while a typical spore is about one ten-thousandth of an inch.)

When deciding which filter to use, you should know what quantity of dust and spores a filter can trap, in addition to knowing that it can exclude flies. Testing is not an easy task on commercial farms. Instead, ask the supplier to provide test data concerning particle size removal using a standard ASRAE (American Society of Heating, Refrigerating, and Air-conditioning Engineers) test. These are standard tests of which the most common is the "weight arrestance test," that uses standard test dust and will show the percent efficiency of the filter media at removing particles by weight. Typical fibrous filters have efficiencies of 60 to 80 percent, with some reaching 90 percent of the test dust trapped. HEPA filters are the most effective at removing small particles and commonly have efficiencies greater than 99.97 percent. This is the percent of the total weight of the dust that is trapped and does not relate to a specific particle size. A filter with a rating above 60 percent efficiency usually will remove all particles of less than five microns.

The higher the efficiency of the material, the better the dust exclusion, but high-efficiency filters will cause more air restriction than low-efficiency models. In general, higher-efficiency filters will need more filter surface area to allow the fan or blower to deliver sufficient air. Typically, the pressure drop for the filter should not exceed 1.0 inch of water. Filters must be tested at the mushroom house to ensure they do not restrict the air too much. Also, the filter must be able to withstand the rigors of mushroom house installation. Paper filters are very efficient but cannot be used in the moist atmosphere of a mushroom house. Therefore, filters made from glass fiber or other synthetic materials are preferred. Some manufacturers also coat filters with a viscous material known as a tackifier to aid in trapping particles.

Once a suitable material is found and attached to a door, filter frame, or other opening, the edges must be sealed. Gapped, loose, or bunched edges of filters or netting are excellent entranceways for flies, and the filter is rendered useless if a good seal is not made there. Flies are tenacious in their attempts to enter a mushroom house, and they have nothing to do all day but look for ways to get inside. They can smell compost and will mill about the outside until they find a way inside. Insects will follow the path of least resistance; a fly walking along a wall will not climb around or over the seam, but will go under it if there is an opening between the material and the wall. The simplest method to seal the edges is to fold over the material and staple the edge directly to the doorjamb. Replaceable boards attached to the doorjamb as a stapling surface will extend the life of the jambs. Narrow slots into which the material is pushed or even Velcro can create effective seals. Regardless of the method selected, a good seal is paramount. Workers performing the installation must be trained to make sure the material is sealed and not simply installed.

Additionally, an adhesive like Tangle Trap improves the edge seal. Flies snared by the adhesive not only are incapable of crawling under a filter but also are prevented from finding other cracks. This is more important than it may seem, since you probably never will succeed in sealing all of the cracks in a building. Spray adhesive on netting and filter seams, doorframes, fan openings, and filter frames.

There are times when an entranceway must be opened for a very good reason early in the crop cycle, for example during the three days before and after spawning. Unfortunately, this is the most critical time for fly control in the crop cycle. Fresh Phase II compost is very attractive to the female sciarid fly, and the compost is very susceptible to green mold colonization at this time as well. (This will be discussed thoroughly in later sections.) But this is also the time when spawn and supplements, spawning equipment, and other items must be brought into or taken from the rooms. Often, a portable air conditioner must be installed in one of the doors to help cool the room. When performing these tasks, limit the time the door is open and take precautions to prevent infestation or contamination.

When employees bring equipment or materials into the growing rooms, they must keep doors closed when not actually entering or exiting the room. Train them and remind them constantly. Teach your employees the importance of keeping doors closed. On the other hand, an automatic door closer, even something as crude as a spring, rubber strap, or counterbalance, will help significantly to prevent fly entry if open doors are a problem on your farm. Also, train employees to recognize and eliminate straight flyways. If a breezeway door is open, all room doors should be closed. For example,

when spawn is transported to a room, it first should be unloaded into the breezeway via the entrance door while all room doors are closed. Once all the boxes are inside the breezeway, close the outside door and open one room door to put the spawn into the room itself. While the door to the outside is open, direct fans at the doorway to help prevent dust from drifting inside and to break up the flight paths of any flies that may try to pass through.

Portable air conditioners required at this time usually are installed in an outside door presenting additional exclusion challenges. Until installation and sealing are complete, flies, spores, and dust have a direct and unimpeded path into the growing room. Installation, therefore, must be quick. The unit should be on wheels, and methods should be devised to get it installed and sealed quickly. Attach a sheet of plywood with a border of foam rubber to the front of the portable unit so it can be wheeled against the wall and sealed at the same

time. No tools should be required during installation. Simple, hand-tightened turnbuckles can draw the foam tight against the wall and jamb. Pay close attention to mated surfaces after installation. Improper installation provides entry points for flies. Lastly, spray the edges with fly trapping material.

Though not as susceptible to disease organisms as cooldown and spawning, the casing operation and casing preparation can have pest problems. Phorid flies are attracted to actively growing mycelia, and *Verticillium* spores can infest the casing. Take the same precautions during these operations as you use during spawning.

Exclusion also involves controlling the movement of people and equipment. Anyone who has been in older rooms—harvesters, maintenance people, supervisors—must not be allowed to enter new rooms they could infest by bringing in contaminated casing or

Figure 12. Movement of employees between "clean" and "dirty" areas must be controlled.

compost, spores, flies, or mites (Figure 12). Harvesters should have lunch and break areas separate from employees who work in clean areas. Always assume that areas are contaminated if frequented by harvesters or people working in other dirty operations such as Phase I compost filling. These areas must be sanitized periodically, and of course are always off-limits to people who work in clean areas.

Equipment used in older rooms—hoses, spraying equipment, harvesting equipment—should not be used in clean rooms. Make separate clean and dirty room equipment available. If a piece of equipment must be used in both clean and dirty rooms, sanitize it thoroughly before using it in a clean area. For example, always use spraying equipment for pesticide applications in clean rooms first and then in progressively older rooms. Of course, the equipment must be sanitized before use in clean rooms the next day.

Exclusion continues to be important toward the end of the crop. At this stage, instead of trying to keep pests out, they must be kept inside the growing rooms. Exclusion now is more aptly called containment. Flies are actively seeking ways out of the growing rooms, looking for fresh compost or growing mycelium, and are most likely carrying pathogenic organisms. Though not as critical as the employees in the spawning operation, harvesters also must be trained to keep doors closed. Filters must be kept intact. Filter exhaust air as well, to prevent expulsion of spores and flies into the outside air.

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A. Specific Control Techniques

2. Cultural Control

Phillip S. Coles
William Barber

Mushrooms are saprophytes, part of a group of decaying organisms that are nature's "janitors." Together with other decaying organisms (various bacteria, molds, etc.), mushrooms (fungi) eliminate dead material in nature. If not for the action of these "janitors," the world would be buried in dead plant and animal material.

In nature, it might not matter which organisms consume dead leaves or a fallen tree, but in trying to grow a specific fungi, the environment must be manipulated. This is possible with specific cultural control techniques. Proper use of cultural controls can manipulate growing environments to favor the cultivated mushrooms and to discourage competitor organisms or pathogens. Mushroom mycelium can overcome a weed mold, for example, if the weed mold is put at a disadvantage. No other means of control are needed if environmental manipulation is successful or the competing organisms are weakened to the point that they become susceptible to other controls such as pesticides or biological agents.

Many common IPM practices are not normally thought of as cultural controls. Phase I and II composting, for instance, are good examples of cultural controls. Mushroom mycelium will grow readily on many of the materials used to make compost if those materials are autoclaved (sterilized in a sealed container)

and mushroom mycelium is aseptically transferred into them. Composting *is* required to make the material pliable so it will hold sufficient water to sustain the mushrooms through the crop and to create enough density to allow trays or beds to be filled with the desired dry weight at a reasonable depth. However, the most important reason for composting is to make the materials selective for mushroom mycelium (Figure 13).

If mushroom mycelium is added to uncomposted materials—materials that are not selective for it—competitor organisms will quickly take over. They are able to grow much more quickly than mushroom mycelium and will exclude the mushrooms through rapid growth, heat production, or production of antibiotics (chemicals that prevent the growth of other microorganisms such as fungi and bacteria). Most microorganisms produce antibiotics as a form of chemical *warfare* to control their territories. (Penicillin is a good example of an antibiotic produced by a fungus, in this instance by *Penicillium* mold.) Composting changes the raw materials, making them more attractive to the mushroom mycelium and allowing the mushroom to outcompete the competitors. Composting, therefore, is a form of pest control, and as such is part of any good IPM program.

Figure 13. Making selective compost is one of the most important components of cultural controls and IPM.

In nature, a succession of organisms work together to decompose organic matter, and a similar process takes place when compost is made and mushrooms are grown. Mushrooms are simply one of the organisms in the succession, though it is imperative that mushrooms are introduced at the right time in the sequence. When raw materials come to the compost wharf, they should be dry to prevent microbial action and decay. Once they are wetted, the composting process begins. The first organisms to grow are the opportunists, fast-growing microbes that release a lot of energy, CO_2, and water. This causes the compost to become hot and explains why it requires abundant oxygen. If composting is done correctly, microbes will produce ammonia and concentrate simple carbohydrates into larger molecules. The more complex carbohydrates are saved for a later time when the mushroom mycelium is introduced, because unlike many of its competitors, mushroom mycelium is capable of producing enzymes that can break down these larger molecules. The presence of these large molecules, after a properly managed Phase I, is one of the characteristics that makes compost selective for mushroom mycelium. Another characteristic is the conversion of ammonia, by the action of compost microflora, to microbial protein. This stage is Phase II composting.

It is not enough simply to allow composting to proceed uncontrolled. If composting materials are too dense or too wet, air will be excluded and anaerobic organisms will begin to grow. These organisms will ensile the compost. This is desirable in a silo where materials are placed with the intention of excluding oxygen and growing organisms that will ensile the material so it will "keep" and can be fed to cattle at a later date. However, this is completely undesirable when producing mushroom compost. Anaerobic organisms in mushroom compost make the compost selective for other types of organisms by "keeping" the nutrients in the wrong form and by producing anaerobic compounds that are difficult to break down during the composting cycle. Regardless of when they are produced in the composting process, anaerobic compounds are toxic to mushroom mycelium if they remain after Phase II.

Figure 14A. Relation of temperature to growth rates of a typical psychrophile, a typical mesophile, a typical thermophile, and two different hyperthermophiles. The temperature optima of the example organisms are shown on the graph.
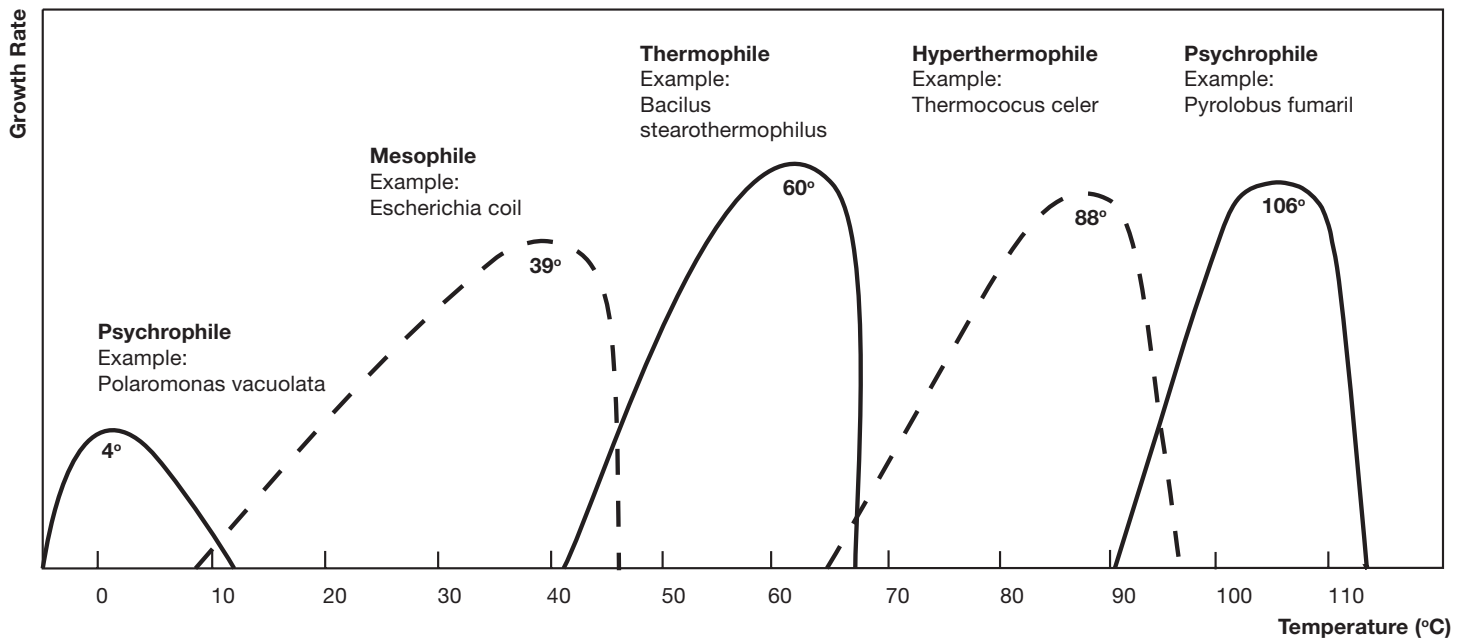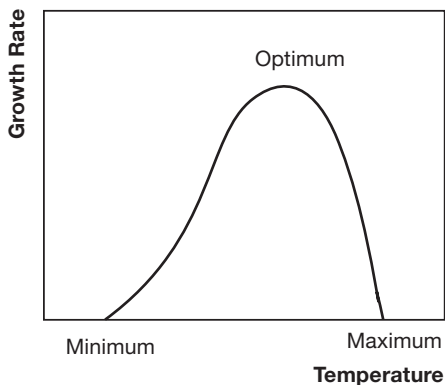

Figure 14B. The effect of temperature on growth rate.

Temperature is a good indication of what is occurring inside the compost pile (Figures 14A and 14B). High temperatures indicate that aerobic composting is taking place. Low temperatures, on the other hand, can be an indication of anaerobic composting.

Compost formulation is an important part of IPM. If the formulation is incorrect, excess nutrients will be left in the compost. If nitrogen supplementation is too high, for instance, excess ammonia will be produced. Ammonia above .05 percent is toxic to mushroom mycelium and promotes the growth of undesirable fungi like *Coprinus*. (Section II.C.3.) Conversely, supplement compost with too little nitrogen and simple carbohydrates will remain (residual) in the compost after Phase II. Excess carbohydrates promote the growth of fast-growing competitor organisms like *Aspergillus*, which will overtake mushroom mycelium.

Length of composting in either extreme can have results similar to under- and over-supplementation. If Phase I is too short, excess carbohydrates will remain. If it is too long, energy will be depleted until not enough remains in the compost for conversions required in Phase II.

Phase II

In Phase II, an entirely different set of organisms grow. These organisms consume ammonia and convert it to protein. Ammonia contains a nitrogen molecule that is essential to protein formation, and the Phase II organisms are capable of obtaining this molecule from ammonia. Later, these organisms will become the protein source for mushroom mycelium. They also use the remaining simple carbohydrates left over from Phase I as fuel. The importance of the Phase I formulation should be apparent.

Just as Phase I can fail due to management practices, incorrect temperature management in Phase II can spell disaster for pest control. If the temperatures are too low or too high, ammonia-converting microbes will be unable to perform effectively. If temperatures in the compost are too high, for instance, Phase I conditions will recommence and ammonia production will continue. Furthermore, the organisms that thrive at high temperatures and produce ammonia will use some of the simple carbohydrates the Phase II ammonia-converting organisms will need. There will not be enough simple carbohydrates (energy) for these organisms to convert the ammonia. They literally will run out of food, and the unconverted ammonia will cause problems for the mushrooms. If temperatures are maintained at levels that are too low, ammonia-converting microbes will not survive, since they require specific temperature ranges to flourish.

Pasteurization

Pasteurization is another critical pest control step in Phase II. Compost can contain many types of pathogenic organisms. Nematodes are the most common, but other types of molds and their spores also are present. Proper pasteurization ensures their destruction. Compost pasteurization serves the same purpose as the pasteurization of milk. Temperatures are raised sufficiently and for an adequate time to ensure the destruction of pathogens, but are low enough to allow the survival of beneficial microflora. By pasteurizing rather than sterilizing, which is done at temperatures that will destroy all organisms, surviving beneficial microflora help to exclude pathogens that later may be introduced to the growing room. The microflora exclude pathogenic organisms by "tying up" sites, or prohibiting pathogens from obtaining substances they require, such as food. They also are capable of producing antibiotics, which can destroy pathogenic organisms. The microflora population remaining after pasteurization is important, therefore, to the successful completion of Phase II composting, but also serves as direct competition for invading pathogens.

Temperature and Humidity Control

During spawn run, optimal temperature is again very important. If the compost temperature is too low, mushroom mycelium, obviously, will grow slowly. Although growth of pathogenic organisms also will be slowed, their growth will not be retarded as much as that of mushroom mycelium. High temperatures are a greater problem. They not only will weaken or kill mushroom mycelium, depending on the temperature ultimately reached, but also will promote the growth of heat-producing competitors. These make temperature control more difficult. To make matters worse, dead mushroom mycelium becomes a source of simple sugars, providing food for the competitor organisms.

It is not adequate to rely on a good "average" temperature. The average of 110°F (43°C) and 40°F (4°C) is 75°F (24°C), but neither temperature is conducive to optimal spawn growth. Hot areas must be located and controlled before they spread and cause severe localized damage similar to the damage occurring in an entire room that has overheated.

After casing, while temperature remains important for reasons similar to spawn run, there are additional considerations as the crop progresses. If temperatures are raised to promote early maturation of mushrooms, other organisms such as *Verticillium* can increase their populations very rapidly. Although the elevated temperatures may cause the mushrooms to mature more rapidly, *Verticillium* will spread even faster.

Relative humidity control, as most growers realize, is important for overall mushroom quality, but it is also an important part of IPM. A film of water on wet mushrooms provides an ideal habitat for *Psuedomonas tolassi*, the causal agent of mushroom blotch. Maintaining dry mushroom surfaces is the most effective method for preventing blotch, and relative humidity control is one tool for accomplishing this. (See Bacterial Diseases, Chapter II.C.4.)

Shortening Crop Cycles

Any technique that shortens the length of the crop cycle aids pest control by reducing the amount of time pathogenic organisms have to reproduce. The strategy is to complete the harvest and pasteurize the room before pest populations can damage the crop. The benefits are twofold: first, when pest organisms enter a growing room, they do not have sufficient time to reach economically injurious levels within that crop. Second, it reduces the amount of innoculum on the farm for new crops in other rooms. This applies to arthropod pests as well as fungal pathogens.

There are many ways to reduce the time needed for the cropping cycle. Most important, run the growing rooms properly from the start. Low temperatures or mechanical problems in spawning or casing can delay the onset of picking and expose the room to excessive increases in pest populations. Phase II rooms must be brought into conditioning range without undo delay. Cooldown-to-spawning times should be kept to a minimum, and proper spawning rates must be used to ensure complete colonization in a minimal amount of time. During cropping, remaining mushrooms from each break should be stripped to help the next break come in more quickly.

Growing techniques that shorten crop cycles also should be considered, such as adding CAC-ing (Compost At Casing) to the casing layer and reducing the number of breaks. Through-spawning and supplementation are examples of methods used in the past to shorten crop cycles.

Sanitation

Sanitation is essential for controlling mushroom diseases and arthropod pests, because it will slow the spread of pathogenic organisms as well as lower their overall populations in the mushroom-growing environment. The place to start is outside the growing rooms. Roads and the immediate vicinity of mushroom houses should be paved with concrete or macadam, since dust is an excellent carrier for the sticky spores of *Verticillium* or *Trichoderma*. Areas around growing rooms, tunnels, and other sensitive locations should be kept free of dirt. In dry weather, water to keep dust to a minimum. These areas, also, should be kept free of clutter. Debris provides hiding places for flies, sheltering them from inclement weather. Mow grass to reduce areas where flies can hide from sun, frost, or rain.

The walls and floors of rooms must be washed and sprayed with sanitizers to ensure all pathogens are destroyed. Steam pasteurization within a room is not sufficient to ensure these surfaces are free of pathogens, since the walls and floors act as heat sinks. Heat is conducted through the floor into the ground, which has an almost infinite capacity to absorb heat, maintaining the floor cooler than the room air in contact with it and ensuring such surfaces will never attain pasteurization temperatures. For the same reason, the basement floor of a house always will be cold unless it is insulated.

The importance of washing before sanitizing cannot be overemphasized, because any dirt left on a surface quickly ties up a sanitizer. This renders it ineffective and essentially protects any spores or other potential pathogens from being destroyed.

Hallways can harbor carry-overs from previous crops. All hallway surfaces must be washed and sprayed. Any areas that cannot be sprayed, such as electric panels, must be wiped down with a cleaner and disinfectant.

Once growing rooms are clean, they must be maintained that way. This would be easy if they could be sealed, but people and equipment must enter rooms to perform mechanical operations and monitor the crops. The movement of equipment and people on a farm must be controlled. Equipment, for example, should be separated and color-coded according to department or use to ensure that dirty equipment such as squeegees from filling operations can't be used in clean areas.

Within the room, good housekeeping minimizes the multiplication of pest organisms. Keep growing surfaces free of organic matter such as dead mushrooms, which can serve as food sources for pest organisms. Mushrooms should be picked tight to reduce the chance of spreading spores containing virus particles.

Steam-off is an important part of maintaining low pest populations. During cropping, pest organism populations will increase inside the house and are potential sources of contamination for new growing rooms. Steam-off, or post-crop pasteurization, eliminates these contamination sources. Prior to steaming, growing rooms should be closed, and openings such as those for fans should be closed to prevent the escape of pathogens as the

room is heating up. If fly populations are very high, the room should be sprayed with quick knockdown insecticides to prevent escape once the steam is turned on. After steam-off has started, monitor both compost and air temperatures with probes. Raise the air temperature with live steam to 160°F (71°C) and maintain it there until the compost reaches the same temperature. Begin counting time when the compost reaches 160°F (71°C). The compost temperature should be 160°F (71°C) for at least 5 hours to ensure an adequate kill of all pathogens. If pathogenic organisms are found to be surviving steam-off, wet the casing surface before injecting steam to help with heat transfer through the compost and casing. Also, more time can be added to the steam-off.

A. Specific Control Techniques

3. Biological Control

Danny Lee Rinker

Introduction

Biological control provides the mushroom grower with natural tools to control mushroom pests. Natural anti-pest capabilities of nematodes, wasps, or bacteria are exploited. Biological control also capitalizes on the properties of chemical substances released by the pest or by food on which it is feeding.

Biological control methods offer many advantages over chemical control: the agents have a specific host range, there are no toxic residues, and concern for worker exposure is reduced. Biological control methods can target a pest and reduce its numbers to an acceptable farm operational level. In addition, the control agent may be self-perpetuating, reducing the need for frequent reapplications. Development of resistance is rare.

Biocontrol requires the support of an IPM program to create the conditions under which biological agents can be most effective. For example, the agent may act only against an immature stage of the pest and have little impact on the adult, necessitating that the IPM program call for its use when the immature forms are predominant in the pest population. Or the biocontrol agent may be more susceptible than the pest to pesticides and may be killed or weakened when pesticides are used as part of existing pest control practices. The IPM program would be alert to this possibility. Application of the biocontrol agent would be withheld until chemical residues have dissipated, or a control agent may reproduce more slowly than the pests and never "catch up" once the pest population is high. An IPM program then may include other means, perhaps chemical controls, to lower the pest numbers. The biological system can be left to do what it does best—maintain low pest populations.

An IPM program can help the farmer manage other limitations of the biological control method—longer time to peak effectiveness, incomplete elimination of the pest, cost, and inability to overcome the overwhelming pest pressures resulting from poor housekeeping—by calling for other control measures when they in turn are most effective. And, because biological agents may have detrimental effects on the mushroom crop if applied improperly or when their use is not warranted, an IPM program must be in place to ensure that the biological agent serves the grower's needs while avoiding reductions in crop yields or quality. When supported by an IPM program, biological control methods can expand the mushroom farmer's arsenal of pest control weapons.

Biological control programs in the mushroom industry are currently being used for nuisance flies on compost wharves, sciarid flies, and blotch disease. Additional potential agents for biological pest management in the mushroom industry are cited.

Nuisance Flies on Compost Wharves

The house fly *(Musca domestica)* and the stable fly *(Stomoxys calcitrans)* are common to compost wharves. An effective biocontrol method augments the number of naturally occurring Pteromalid wasps. These tiny wasps are parasites that attack the immature pupal stage in the fly's life cycle. The wasps are nocturnal and do not sting. Commonly used species are the *Spalangia endius, Muscidifurax raptor, M. zaraptor*, and *M. raptorellus*.

The Pteromalid wasp has a life cycle similar to other insects: egg, larva, pupa, and adult. Most of this wasp's life cycle occurs within a host that provides nutrition and protection for all stages other than the free-living adult wasp. House flies and stable flies are among their potential hosts. The adult wasp lays her eggs into the nuisance fly's pupa. The immature parasites consume the host's tissues from the inside, and adult wasps "host feed" on fluids from the outside, preventing the fly from developing into a healthy adult. The wasp's life cycle requires two to four weeks. Under optimal conditions, the parasites can reduce the nuisance fly population in 4 to 6 weeks. Complete elimination of flies usually is not possible, especially where there is migration onto the farm from off-site locations.

Parasitic wasps must be integrated within a pest management program. Reducing conditions favorable to breeding helps to limit fly populations. Flies will reproduce in moist organic matter; therefore, promote good drainage, remove seepage, and minimize standing water to lower the number of nuisance flies. Good sanitation on the wharf, rotation of raw materials, and removal of spent substrates from the farm also will expedite fly management. Routine fly monitoring is essential to evaluate the necessity and effectiveness of a management program. Release rates of parasites are dependent on fly numbers, environmental and climatic conditions, migration from off-site locations, and chemical controls.

Wasp release early in the season is a good strategy, since the fly pests have certain survival advantages over the wasps—greater reproductive capacity, ability to fly greater distances, and greater resistance to pesticides. If the number of adult flies is too high, bait trapping can initially reduce it. Once the adult fly number is lowered, the wasps can be used to keep the pests under control.

Sciarid, Phorid, and Cecid Management

Mushroom fly pests are a consistent problem for growers. The three fly groups most commonly encountered are the sciarid fly *(Lycoriella mali),* the phorid fly *(Megaselia halterata),* and the cecid fly *(Mycophila speyeri*, *Heteropeza pygmaea).* The sciarid larvae attack compost, spawn, mycelia, pins, and mushroom stems and caps. The larvae of phorid flies feed on mycelia, causing depressed crop yields. Cecid larvae feed on the mushroom stems or gills, reducing marketable yield. Sciarid and phorid adults carry disease organisms into the crop. Mushroom flies are discussed in greater detail in Chapter II.C.1 of this manual.

Nematodes as Control Agents Against Mushroom Flies

Mushroom flies are good targets for biocontrol with beneficial nematodes. More information on nematodes, especially those that negatively affect the mushroom crops, can be found in Chapter II.C.5 of this manual. Beneficial nematodes, those that can play a role in biological control, are covered here.

Howardula husseyi as a Control Agent Against Phorids

Howardula husseyi is an endoparasitic nematode that occurs naturally in the phorid population. This nematode lives both in the compost and in the fly. It has a six- or seven-stage life cycle: egg, four or five immature larval stages, and adult. Some of these immature-stage larvae are free-living, while others are parasitic. Both adult male and female phorids are commonly infected with nematodes.

When the female phorid attempts to lay eggs on spawned mushroom compost, she also deposits second-stage nematode larvae. Female and male nematode larvae develop and mate while in the compost. The fertilized fourth-stage females ("infectives") enter the phorid larvae or young pupae and develop into adults while in the phorid body cavity. While inside, they also deplete and disorganize the fly's food reserves and lay eggs. After the eggs hatch, the young nematodes work their way through the phorid ovaries into the oviducts. When the female phorid attempts to oviposit, she discharges the nematodes.

While in the phorid, the growing and developing nematodes reduce the phorid egg production by 50 to 100 percent. Under controlled lab conditions with parasitism increasing uncontrolled, phorid populations can be virtually eliminated within five fly generations. Commercialization of this nematode species has not been successfully achieved.

Steinernema feltiae as a Control Agent Against Sciarids

A beneficial entomopathogenic nematode, *Steinernema feltiae*, has been impressed into service as a biocontrol agent against sciarids. This nematode species carries bacteria that are deadly to the sciarid fly. Bacteria live within the gut of the nematode and are released once inside the host. The nematode life cycle includes the egg, four juvenile stages, and the adult. The third juvenile stage generally enters the third or fourth larval stage of its host through natural body openings like the mouth, anus, or spiracles, or it may go directly through the body wall. Once inside the host, the nematode makes its way into the body cavity of the insect larva and releases the bacteria. These bacteria rapidly kill the host within 48 hours by blood poisoning. The immature nematodes feed on

the new bacterial cells and host tissues and then develop into adults. The adult nematodes reproduce in the host. Young nematodes, finding the food supply depleted, will exit the cadaver.

Infective-stage nematodes can be applied to the casing material at casing or later during the crop. The nematodes do not feed in the compost or casing. They can be responsible for high levels of mortality among *L. mali* larvae. Lab trials have shown *S. feltiae* to cause mortality levels up to 100 percent. On-farm trials have attributed lower, but significant, reductions in fly emergence (e.g., 66 percent fly emergence) to the pathogenic effects of *S. feltiae* when applied at rates of 81 million nematodes per 100 m^2. In order to achieve higher mortality, current supplier recommendations are 300 million per 100 m^2.

Microorganisms as Biological Control Agents of Mushroom Flies

Bacillus thuringiensis subspecies *israelensis* (Bti) is a bacterium that is used widely in biocontrol. The bacterium produces both a protein crystal and a spore. Once eaten by the larva, the crystal degrades in the alkaline gut of the insect. The insect's gut subsequently becomes paralyzed, and larval death occurs within 48 hours.

Field trials have demonstrated control of phorids and sciarids when Bti was applied either to compost or casing. Small-scale research trials with one formulation have demonstrated that a compost application could provide 85 percent sciarid control. An application to casing at a lower formulation rate manifested a 70 percent control level of sciarid larvae. Excessive mycelial growth on the casing or reductions in yields may occur from a casing application. Bti appears to be more effective against younger rather than older sciarid larvae.

Mites as Control Agents against Mushroom Flies

A predator consumes its prey, either partially or entirely. One such predator in the mushroom crop is the mite *Hypoaspis miles*. This mite will prey upon larvae of cecids, phorids, and sciarids. These mites are commercially available for use in the greenhouse industry, but none are being used at this time in the commercial mushroom industry. The mite is less than 1 mm in size and light brown in color. The life cycle is complete in about two weeks. In small-scale experimental trials, sciarids were controlled at 96 percent using 750 mites per m^2. However, field trials in Ontario were not as dramatic, with less than 30 percent control of sciarids. The mites do not suppress mushroom yield, nor are they found on mushrooms. Mites have several advantages. They are mobile and can easily search for prey; mushroom growing conditions are suitable for their reproduction; and they can live for several weeks without food. Despite the low control rate thus far, predatory mites do have potential for successful integration into biocontrol of mushroom pests.

Fungi

Some fungi are capable of invading the bodies of flies. The pathogen spore or mycelium penetrates, develops, and kills the host. After death of the host, spores are produced on the cadaver's surface. These spores then will infect others. The development of *Pandora gloeospora* has shown promise for control of sciarids.

Biologically Derived Chemicals Used in Biological Control of Insects

The term "biological control" has come to include the use of chemical substances with control potential. In some cases, insects themselves may be the source of the chemicals that will attract others. Or, substances can be produced by the food source that may either attract or repel insects.

Pheromones

Pheromones are volatile chemicals that help insects find each other or cause some other biologically important response. The compounds are species specific and are detected by the insect in minute concentrations. In a biological control program, these chemicals are used to attract large numbers of the pests (mass trapping) to stop an infestation or, conversely, to confuse the pests so the two sexes cannot find each other to mate. Sex pheromones have been demonstrated for phorids and sciarids. However, commercial trials using synthetic compounds have not been successful.

Kairomones

Kairomones are volatile chemicals produced by a pest's food source that alert the pest to its presence. They could be used as a chemical message to lure pests into traps. The attractiveness of compost to sciarids during Phase II cool-down or of actively growing mycelia to phorids are observed events. However, researchers have not been able to duplicate the phenomenon observed in the field.

Repellants and Anti-Feedants

These chemicals act as a "self-defense" mechanism for the food source. They either repel the pest from the food source before it feeds (repellant) or afterwards (anti-feedant). Calcium oxalate, a byproduct of mushroom mycelial metabolisms, has been found to have repellant activity toward the larvae of sciarids. By itself, calcium oxalate is not an effective control method, but as part of a wider program, it may prove to be useful.

Disease Management

Most biocontrol efforts against mushroom diseases have focused on bacterial blotch. Bacterial blotch disease, caused by *Pseudomonas tolaasii* or *P. gingeri*, has been managed commercially by another bacterium *(P. fluorescens* biovar V). The biocontrol agent acts as a preventative, becoming established before the development of a blotch population. It competitively excludes the colonization and development of the blotch population. Bacteriaphages (viruses that infect specific bacteria, usually killing them) also have been used successfully against blotch. Combining both organisms could be highly effective.

Conclusion

Biological control of mushroom pests is a reality. Parasitic nematodes and wasps are commercially available and are becoming integrated into pest management programs, while other organisms including fungi, bacteria, and mites are being developed. The prospects are favorable for the development of effective biological control agents in the next several years.

A. Specific Control Techniques

4. Chemical Control

Phillip S. Coles

Integrated Pest Management is not synonymous with organic or pesticide-free production, as many people believe. Judicious use of chemical pesticides is an integral part of an IPM program. In IPM, pesticides are not applied on a rigid schedule as they are in a chemically dependent pest control program. They are one facet of a broad *(integrated)* approach to pest management, though one that frequently can be minimized or avoided altogether.

Many pesticides appear to provide superb pest control independent of other control measures. After an application of a contact material, for instance, many dead insects may litter the outside of a plant, tempting complete reliance on such products. After a simple (though not inexpensive) application, the grower feels good. The application complete, there is now more time to devote to the long list of other duties that confronts a grower each day on a mushroom farm. Regularly scheduled pesticide applications, therefore, become appealing.

But using chemical pesticides in this fashion is destined to develop pest resistance to them. Pest organisms readily become resistant to overused chemicals. (See Chapter II.B, Resistance management.) Pesticides in an IPM program, however, are applied as a last resort and are used in accordance with monitoring, established economic thresholds and temperatures, and never on a rigid schedule. Also, pesticides are most efficient if used when they can lower a high pest population rapidly and significantly, providing the grower the opportunity to get the pest under control and possibly saving a crop obviously in peril. Other IPM strategies then can maintain that level of control.

There are two pesticide application techniques on a mushroom farm. One is preventative, and is predicated on monitoring and temperatures, while monitoring exclusively triggers the other. Preventative applications serve the same role as physical exclusion. Instead of making it physically impossible for fly entry, however, a chemical barrier is applied in an attempt to kill the fly before it gains access to the growing room. This is not as effective as physical exclusion, but it is a backup to it if some entry points have been overlooked. Use a contact poison for this application. There are other types of pesticides, but more on them later.

Preventative applications do not replace diligently sealing the growing room. On the contrary, the two must work together. (See Chapter II.A.1, Exclusion.) If a contact spray were used without physical exclusion, only the most resistant flies would be entering the rooms to reproduce. In effect, you would be screening for "super flies."

Only use spray when weather is conducive for fly movement and when significant fly populations exist on the farm. Every farm will be different, and each farm should develop its own procedures dictating when spraying should take place. If the temperature is below freezing outside and your growing rooms are physically separated, flies cannot move from old to new rooms or from the wild population into new rooms. Determine at what temperatures they will move on your farm, and do not use preventative sprays when the outside temperature is below the established figure. (See Chapter II.C.1, Arthropod Pests, for flight temperatures.) You may want to decrease your threshold by a few degrees to add a safety margin.

Assess fly populations in two ways. First, population numbers should be available from monitoring inside the growing room. Have the numbers been high, or are they becoming high? Managers at each farm must decide just what is "high" when referencing monitoring data. Second, growers should have a good feel for fly populations from time spent in the growing rooms. In early crop stages, usually it is not possible to detect flies without monitoring. If you can, you have a very serious problem! But, in the later stages of harvesting, high fly populations are detected easily. A grower must make spraying decisions with this information. An example of a spray-triggering scenario might be a daytime high temperature above 50°F (10°C), obvious fly populations in old harvesting rooms, and spawn run fly counts consistently above 10 flies per day. Obviously, the parameters would vary from farm to farm.

A common use for preventative sprays is applications to the outsides of buildings. Outside spraying, however, has serious disadvantages. It is difficult to get complete coverage around and on top of a building. It cannot be done during inclement weather. The pesticide is exposed to the elements—rain can wash it off—and ultraviolet rays from the sun break it down. There are also environmental concerns. The pesticide is outside where it can contact non-target organisms, and care must be taken not to contaminate streams or other water

supplies. Never spray when there is a chance of drift.

Chemical applications to enclosed areas outside of the actual growing area, such as hallways and lofts, are a more effective use of preventative sprays than on the outer surfaces of buildings. Good coverage is attained more easily; the material is protected from degradation and wash-off; non-target organism exposure is limited; and the percentage of pest populations exposed to the pesticide also is limited. This helps with

Figure 15. Fly light with many sciarid flies on it.

resistance management, since target organisms must penetrate the walls of the growing building before coming in contact with the pesticide. Logical targets for pesticide applications are inside growing rooms, on beds or trays, and on any plastic covering the compost. Of course, pesticides used in growing rooms must be labeled for mushroom use.

Despite exclusion and the use of preventative pesticidal applications, some flies still can gain access to a growing room. To know whether flies are entering a room—and the magnitude of the invasion—you must monitor. The Pennsylvania Fly Monitor provides the best way for commercial farms to monitor fly populations. This monitoring device is simply a black light fastened to a board with a strip of sticky paper on either side of the light (Figure 15). The monitor should be placed in a location proven to collect the most flies of any area in the room. The location will vary from farm to farm, necessitating some experimenting at the outset. Keep in mind that flies tend to be lazy and won't travel far unless it is necessary. Therefore, the monitor usually is placed above the highest bed so the light can be seen from a large portion of the room, as well as near areas of the room where penetration most likely occurs. Flies should be counted daily and this information used to make pesticide decisions. Decisions can be made according to daily or cumulative counts. Daily counts would trigger the use of a knockdown material, an adulticide, while cumulative counts would dictate the use of a larvicide in compost or casting.

Remember, a fly monitor does not catch all of the flies in a room. It traps only a percentage of them. The monitor was originally tried as a control method. It was hoped the flypaper would capture incoming flies, and that would be the end of them. After testing, only a percentage of the flies were caught, and these almost exclusively were females that already had laid their eggs. This meant the monitor was useless as a control technique; but by sampling the population, it gave a relative fly count for the room and therefore was valuable for making pest control decisions.

It is important at this point to distinguish between different types of materials, for this will establish how they are to be used. As stated earlier, contact poisons make very good preventative sprays. They are in place if a fly tries to enter a growing room and are effective against adult flies. A quick knockdown material such as a pyrethroid fog is very useful if flies suddenly appear on the monitor in cooldown or early spawn run. Larvicides and growth regulators are good for mixing with the compost and/or casing to prevent the flies from reaching adulthood and producing another generation.

Type of Material	Uses	When to Use
Contact poison, adulticide	Preventative, exclusional spray.	Prior to air in growing rooms reaching 100° F (38° C), or before cooled compost is brought in a tray or tunnel system.
Quick knockdown, adulticide	Good when large number of invaders are present, or at the end of a crop when you want to prevent them from exiting a room and invading new rooms.	When fly monitors indicate an influx of invading adults at the beginning of the crop, or when the grower feels fly populations in an old room are high enough to threaten new crops. It also works well as a knockdown to prevent flies from escaping the room as steam is being injected.
Growth regulator or similar larvacide	To prevent eggs laid by invading adults from developing into adults, producing later generations.	When cumulative fly counts dictate their use.

Establishing Economic Injury Levels

Pest control decisions must be based on monitoring. Even preventative sprays have a monitor count component. Unfortunately, there are no concrete, scientifically established economic thresholds for mushroom farms. These numbers are somewhat arbitrary and must be determined by the growers at each farm. If extensive scientific studies existed, there still would be dramatic differences from farm to farm, and economic injury levels (EIL) would have to be customized.

To develop individual EILs, the grower must consider the level of potential damage. For example, sciarid flies cause much more extensive crop loss than phorid flies, and can decrease quality by burrowing into the mushroom stems. Therefore, higher numbers of phorids than sciarids can be tolerated. There are additional outside influences. If disease levels are low, a fairly high number of flies may be acceptable. But those same populations can be devastating if there are high levels of *Verticillium* and/or green mold on a farm. The sciarids are very important vectors of green mold, since they are most attracted to the compost during cooldown, precisely the same time green mold infections are most likely to occur. When *Verticillium* is high, only low populations of both flies can be tolerated, keeping in mind that the high activity level of phorids makes them better at spreading the disease. All of these factors must be taken into account when trying to determine the economic threshold triggering a pesticide's use.

An example of an economic threshold for sciarid flies is two flies a day until the end of spawn run, then 10 per day through harvest. Obviously, higher populations can be tolerated as the crop progresses (see Part I, Theory of Integrated Pest Management). This is also an example of a daily EIL. If more than two flies per day appear on the fly monitor, the grower would be justified in spraying a fogging adulticide to kill the incoming invaders from that day.

Cumulative counts determine the use of larvicidal agents added to the compost and/or casing. High fly counts early in the crop would indicate future problems, since a significant number of the invading adults probably will be successful in ovipositing in the compost. A strategy is needed to prevent them from producing subsequent generations. If a material is added to the casing layer, there is more than adequate time for the grower to make a control decision. For example, if experience had shown that significant fly damage would result if more than a cumulative total of 200 sciarid flies (a cumulative EIL) had entered a growing room prior to casing, then a larvicide should be added to the casing. On the other hand, if a material must be added at spawning, there is not as much time to make a pest control decision. In this instance, the material has to be applied before the spawning machine mixes the spawn with the compost, so the cumulative EIL would be a specific number of flies on the monitor up to the day of spawning or possibly until spawn broadcast. If a larvicide is added to the compost—either on the compost wharf or at fill—it is more similar to a preventative spray than a spray based on an EIL; therefore, preventative spray criteria would determine whether or not the larvicide was used at this stage.

Formulations

There are several types of formulations used for pesticides, each having its own advantages and disadvantages. Some pesticides are available in more than one formulation, so their use can vary depending on the situation. Most pesticides are available in only one formulation, so their effectiveness must be weighed against the advantages of the formulation.

Classes of Pesticides

There are several classes of pesticides. Some, like the organophosphates and carbamates, contain some of the original pesticides such as DDT. The more modern materials such as insect growth regulators (IGR) are becoming more common. They also are safer than the older materials and much more specific in their range of effectiveness. The grower doesn't have much choice in what class of pesticide is used, since only those registered can be used. Work with your sales representative to decide which ones are best for your application.

Formulation	Advantages	Disadvantages
Emulsifiable Concentrate (EC) A liquid formulation, oil that makes an emulsion when mixed with water.	Easy on spray nozzles; can be used with small amounts of water; easy to suspend in water; very little residue left in bottom of tanks. Not dusty.	Cannot be mixed dry.
Wettable Powder—pesticide is mixed with clay carrier used for suspending in water.	Can be mixed dry.	Abrasive to nozzles. Difficult to keep suspended in water; much can end up wasted on bottom of tank. Dusty.
Flowable—a wettable powder that has been mixed with a liquid to make handling easier.	Reduced dust as compared to a wettable powder.	Still abrasive to nozzles.
Dust—a pesticide mixed with dust used as a carrier; very dilute compared to a wettable powder.	Coverage can be seen and evaluated.	Dusty, dirty. Leaves a lot of visible residue.

Testing

Finally, no pesticide, regardless of how safe or easy to use, is of any value if it does not kill the target pest or pathogen. Pesticides must be tested, and they should be tested against the specific flies of a given farm. Just because tests show good efficacy against sciarid flies for a material does not necessarily mean it will be good against your farm's sciarid flies. Genetic variation occurs between individuals and between different populations. Generally, when a material is new it will work well, but as it is used (or misused) resistance develops, at different rates in different populations. The only way to be confident in a pesticide's efficacy on your farm is through testing.

Some testing is easy to do and can be done at the farm level, while other tests are too difficult and require a professional. The expertise of the personnel on a given farm will dictate how much if any of the testing can and should be done there.

There are many simple ways to test pesticides. Fungicides are difficult to test because of the difficulties of working with the various pathogens. Generally, insecticides are relatively easy to test. The easiest are the quick-knockdown fogging materials. Make a cage using fly netting that will prevent the flies from escaping, but that also will allow air movement. Place some flies into the cage and hang it inside a room that is scheduled for fogging. After the room has been aired out, count the number of dead and surviving flies.

There are several ways to evaluate contact materials. It can be as simple as placing wood blocks in an area that is being sprayed. Remove the blocks, place them in a container, and add flies to the container. If the flies die, the material works. Also, a lid to a container can be fastened to a wall or ceiling, or simply placed on the floor. After it has been sprayed, fill a container with flies and attach it to the lid to see how the flies do. This involves a little more risk, so avoid taking flies into the growing room. Reserve this method for outside sprays.

Remember that flies are relatively fragile and do not live long, whether they are exposed to pesticides or not. So you always should have a control group for purposes of comparison. If the unexposed flies die, they may have been mishandled. Try the test again.

Once everything is in place to perform the test, flies must be collected. They are very small and fragile, and collecting them is no easy task without the right equipment. The best way to collect flies is with an aspirator. Aspirators are available commercially, some with battery-operated pumps. These are useful if you are collecting large amounts of flies on a regular basis, but you also can fashion an inexpensive aspirator from some very inexpensive lab materials that will suit the purpose at almost any mushroom farm. All that is needed is a flask or bottle with a rubber stopper that has two holes. In each hole is a short piece of glass tubing with rubber tubing attached. One of the pieces of glass tubing will have a piece of netting over the end inside the jar. To collect insects, suck on the end of the filtered rubber/glass tube while holding

the other rubber tube near the insect. It will be pulled inside of the jar for later use. Be sure to place the netting over the end of one tube or you may end up eating your samples!

Conclusion

Pesticides are an important part of any IPM program, but they also have drawbacks and should be used as a last resort after other types of controls have been put in place. They must be used in a responsible manner, not only from a safety and environmental standpoint, but also to ensure their continued effectiveness. A plan must be devised using monitoring and economic injury levels. The safest and most effective materials and formulations must be used, in the proper manner, and their effectiveness ensured through testing.

B. Pesticide Safety

Susan Whitney

Laws Regulating Pesticide Application

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires certification and licensing of users of restricted-use pesticides. Certification documents the fact that applicators/handlers know how to use pesticides safely for themselves, the public, and the environment. There are two categories of applicators: private and commercial. A private applicator is a person who uses or supervises the use of pesticides for the purpose of growing an agricultural commodity such as mushrooms. The application can be done on property owned or rented by the applicator or the applicator's employer. A commercial applicator is a person who uses or supervises the use of pesticides on a "for-hire" basis. State pesticide inspectors with both the Pennsylvania Department of Agriculture and the Delaware Department of Agriculture routinely conduct on-site use observations to ensure that applicators are handling pesticides correctly. Review the safety tips below in preparation for the pesticide inspector.

The Worker Protection Standards (WPS) cover workers and pesticide handlers/applicators in mushroom production. (Workers are those employees who do any kind of work that would bring then in contact with surfaces that have been treated with pesticides in the past thirty days.) All pesticides used in mushroom production must have an "Agricultural Use Directions" statement on the label. Read this statement to ensure that you are complying with the law. For specific details on WPS, consult the "EPA How-to-Comply Manual." The WPS requires employers to provide the following for both workers and handlers/applicators:

1. Information at a central location that includes a WPS safety poster; the name, address, and telephone number of the nearest medical facility; and the name, date, time, restricted entry interval (REI), and application site of pesticides recently applied.

2. Training for workers and handlers unless they are already certified applicators.

3. Transportation to an appropriate medical facility, as well as pesticide use information if a pesticide illness occurs.

4. Decontamination sites within $1/4$ mile of all workers and handlers. Supplies must include water for routine washing—one gallon per worker and three gallons per handler (in some cases, eye flush water must be immediately available for handlers); plenty of soap and paper towels; and clean coveralls for handlers.

The WPS requires employees to provide the following for handlers/applicators:

1. Personal protective equipment (PPE) required by the pesticide label (Figure 16). Employers must confirm that all equipment is clean, is inspected for damage, and is working properly. PPE must be stored away from pesticides.

2. A pesticide-free area for changing clothes.

3. A decontamination site for washing after handling tasks and at mixing/loading sites. Employers must monitor handlers who are using fumigants or any pesticide that has a skull and crossbones on the label.

4. Specific instructions on the pesticide label, including how to use application equipment. Employers must inspect and maintain application equipment. Employers must provide access to labels.

Figure 16. Applying pesticides using typical Personal Protective Equipment.
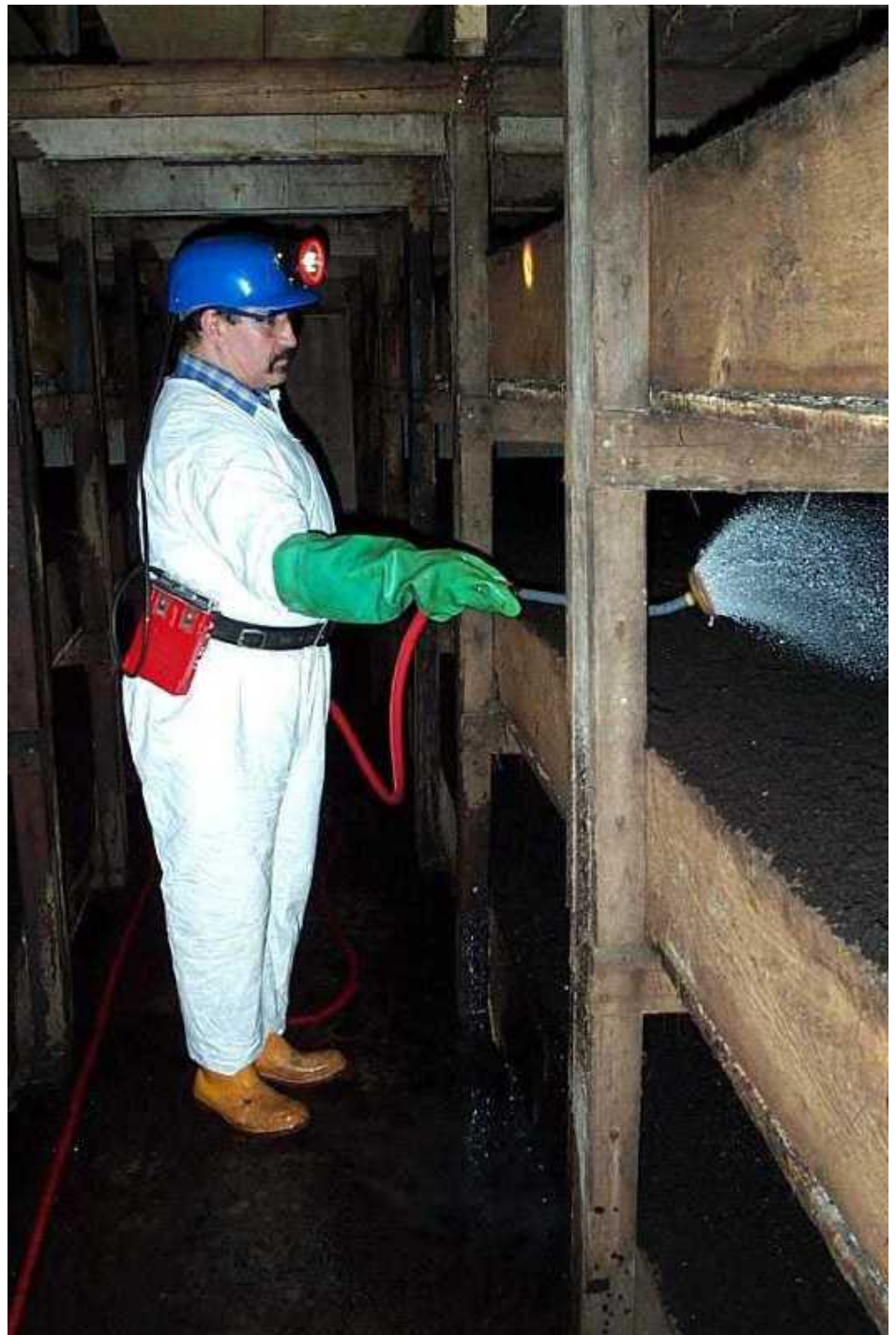
The WPS requires employers to do the following for workers:

1. Notify workers about applications and areas under REI. Employers must post written signs that meet EPA standards. Some products may require both signs and oral warnings.

2. Keep workers out of areas where pesticides are being applied.

3. Keep workers out of areas under REI, except for early-entry exceptions.

Mushroom producers also may have to comply with the Occupational Safety and Health Administration Hazard Communication Standard. This law requires employers to make Material Safety Data Sheets on all hazardous chemicals in the workplace, not just pesticides, available to employees.

The Federal Record Keeping Regulations require that private applicators keep application records of restricted-use pesticides. Records must include the pesticide name, EPA registration number, total amount of active ingredient applied, size of area treated, commodity, location of application, and certified applicator's name and number. Records must be made within 14 days of the application and kept for 2 years in an easily retrievable format. They must be surrendered to medical personnel upon request. Pennsylvania requires these records to be kept for 3 years.

The Worker Protection Standards cover both restricted-use and general-use pesticides. They require producers to keep application information in a central location where workers normally congregate. Information must include the pesticide name, EPA registration number, time of application, and re-entry date and time. Pennsylvania also requires records of the formulation and rate of application for all uses of any pesticide with an REI on the label. Records must be made before the application and kept for 30 days following the REI. This form satisfies both WPS and the Federal Record Keeping Regulations.

Safe Handling of Pesticides

Pesticide labels should be read at least five times: before buying a pesticide; before storing a pesticide; before mixing and loading; before applying the pesticide; and before disposing of the empty container and/or unwanted product.

Keep in mind that *the label is the law!* It is a legal document. If label directions are not followed, the law has been broken—an action that may warrant fines and/or penalties. The label tells how toxic the pesticide is, what PPE to wear, and how to protect the public from exposure and the environment from contamination. The label also tells how, where, and when to apply the product and what pests are controlled.

Probably the most important words on the label are the signal words, which indicate how toxic the product is to the applicator: Caution—least toxic; Warning—moderately toxic; Danger—most toxic. Remember this equation: Risk = Toxicity x Exposure. Your risk of being poisoned by a pesticide is equal to the toxicity of the product times your exposure to the product. Never use a product with a danger signal word if a product with a warning or caution signal word will get the job done just as well. The product with the danger signal word will not kill the pest any faster, but it will be more hazardous to the applicator's health.

Pesticides can enter the human body through contact with the skin, inhalation, or ingestion. For protection from exposure to pesticides on your skin, read the PPE statement on the label. Wear the recommended chemically resistant gloves and coveralls. Clean and maintain PPE according to manufacturer directions. Check regularly for signs of wear and tear. The minimum PPE required for any pesticide application is a long-sleeved shirt and long-legged pants. Of the contamination that lands on a person's body during mixing and loading, 98 percent ends up on the hands and forearms. This contamination is avoided easily by wearing long-sleeved shirts and gloves. For protection from breathing pesticide fumes and vapors, read the PPE statement on the label. Wear the recommended respirator and clean and maintain it regularly. Have a respirator fit-test each season. To protect against ingesting pesticide, never eat, smoke, or drink while handling pesticides. Wear a face shield while mixing and loading to prevent dangerous splashes.

If transporting pesticides from the dealer to your place of business, keep the pesticides in the bed of a pickup truck. Never carry pesticides in the passenger compartment of a vehicle. Tie the containers down and carry an emergency spill kit. Long-term storage of containers should be in a locked, dry, well-ventilated facility that is free from temperature extremes. A sign on the door should warn that pesticides are stored inside. In case of fire, emergency personnel need to know that toxic fumes may come from this room. The floor of the storage facility should be made of sealed concrete for easy decontamination after spills. Any shelving should be of stainless steel, as wooden shelves will soak up spills from open containers. Fumes from such spills

will continue to contaminate any visitor to the room.

Place open containers in secondary containers. Disposable "turkey roasters" from a department store work well. Never keep food, feed, seed, or business products in the pesticide storage facility. Absorbent materials like stationary and paper towels will absorb pesticide fumes and contaminate users repeatedly. Never store PPE in the pesticide storage facility for the same reason. Keep a spill cleanup kit handy: broom, dust pan, mop, bucket, bleach and lye (for decontamination), and spill control products. Cat litter will soak up a spill easily. Newer products made of gel flakes will pick up the spill and allow you to transfer it to the spray tank for application. (Gel flakes will not clog applicator nozzles.) This means that it is not necessary to dispose of a valuable pesticide or hire a hazardous waste contractor to clean up a spill.

Proper calibration of application equipment will save money by avoiding product overuse. In addition, calibration prevents loss of commodity from excess pesticide residue. Fill a spray tank with water and put the nozzle in a bucket to collect the spray. Run the sprayer for the amount of time it would take to spray one bed. Measure the amount of spray collected in the bucket. If this is more pesticide than the label recommends for one bed, it will be necessary to move the spray wand faster over the bed. If the amount collected in the bucket is less than what the label recommends for one bed, it will be necessary to spend more time applying the product to ensure adequate coverage.

While mixing and loading, wear the correct PPE. Connect a backflow preventer to the hose to prevent back-siphoning and contamination of the water supply. During the application procedure, make sure that no workers are in the area. Wear the label-required PPE for the application, and let some-one know you are working with pesticides in case of an accident. Clean the spray tank after each use or use a dedicated sprayer.

After the application, triple rinse or jet rinse empty containers. Take the containers to a pesticide container chipping facility to recycle them. Check with the appropriate state department of agriculture to determine availability of chipping facilities. Unwanted pesticide can be disposed of by use on-site, or a hazardous waste contractor may be hired to remove the unwanted product. Avoid the problem of un-wanted pesticide. Buy only what can be used in one season or less. Stockpiling of inventory is not recommended, because the EPA may cancel a product before one that is in storage is used. Likewise, the manufacturer may produce a better pesticide than an inventoried chemical, or stored products may become obsolete on a particular farm because of a change in the farm's pest complex. There are many reasons for buying pesticides in small quantities and using stock quickly, not the least of which is that improper storage and disposal of empty containers and unwanted product can contaminate the environment.

Following pesticide application, shower and put on clean clothing. Wash applicator clothing on-site, or use disposable PPE. If clothing must be taken home, wash it separately from family wash.

In the case of a pesticide emergency, read the information posted at the central facility, which will include the location of the nearest emergency facility. Clipboards with emergency procedures should be kept in pesticide storage facilities and in mixing/loading areas. All workers should be familiar with, and review often, the information posted on the clipboards.

C. Pest Species Biology and Control

1. Arthropod Pests

Clifford Keil

Sciarid Flies

The major insect pest of mushrooms in North America is the sciarid fly, *Lycoriella mali*. These flies are small black insects about ¼ inch (3–5 mm) long with long antennae and gray wings held folded over the back (Figure 17). Females are more abundant and larger than males. Female sciarids have a pointed abdomen that is frequently swollen with eggs, while males have prominent claspers on the end of their abdomen that are used in mating. Females are attracted to lights and frequently can be seen on backlit windows, vents, picking lights, and black light traps. This attraction to light provides the grower with a means to monitor the number of female flies entering the house and emerging from the compost/casing during the crop. Males, on the other hand, are found primarily on the surface of the casing searching for newly emerged females to mate with. Adult flies do not actively feed but may take in some water. The immature sciarids (larvae) are translucent, white, legless maggots that range in length from ⅛ to ¼ of an inch (1–8 mm). The head is large and dark with powerful chewing mouthparts that distinguish sciarid larvae from other insect larvae that might be found in mushroom production houses. The larvae are the feeding stage in the life cycle of this fly.

Figure 17. Sciarid fly and larva.
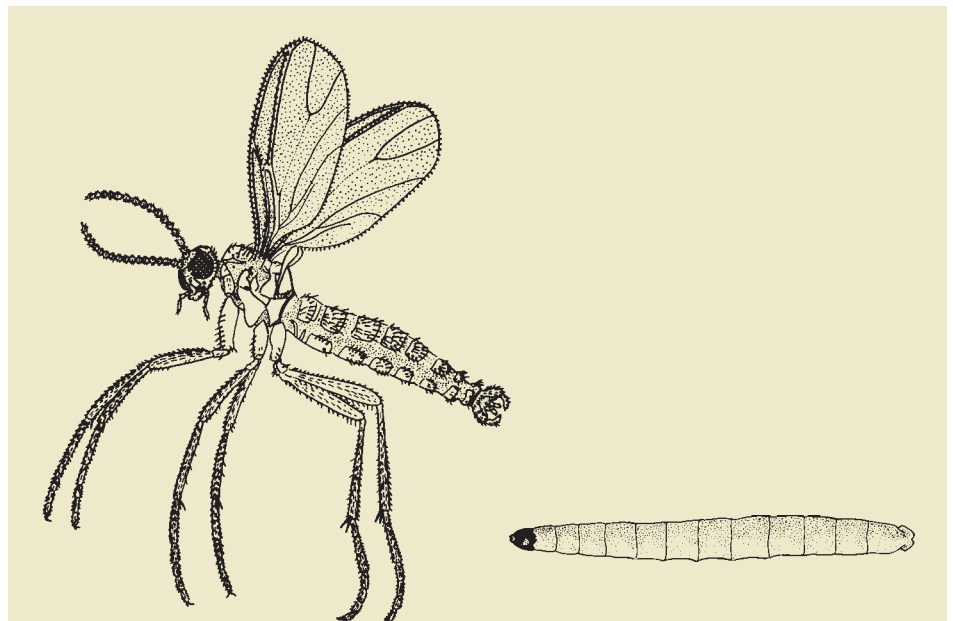
Sciarids like *L. mali* are found naturally in cool shaded woods and areas of dense vegetation. The females seek out spots to lay their eggs where fungi have just begun to colonize the substrate. Accordingly, *L. mali* females invade mushroom production houses as the compost cools down after peak heating and the mesophilic fungi in the compost begin to grow. This invasion continues during spawning. It is essential to protect the crop by placing plastic on the bed surfaces and keeping the doors and other entry points closed during this period. Running black light traps during this period is a good way to assess the tightness of fly exclusion measures and also pinpoint the time of invasion. Female sciarids are capable of finding cracks to enter voids in block walls to find entry into rooms with running spawn. They are very tenacious. Adult *L. mali* prefer cool temperatures and are most active when outdoor temperatures are between 50°F (10°C) and 75°F (24°C). Consequently, the threat of infestation is greatest from March to July and September through late November in most of North America. This threat is diminished during the hottest part of the summer, especially under dry conditions and after three successive frosts.

Once inside a growing room, a female *L. mali* typically will land on compost close to the point of entry to lay her eggs. Depending on how well fed she was as a larva, she may lay up to 150 eggs. Female *L. mali* can be very discriminating in choosing a spot for oviposition. They can detect residues of Dimilin and avoid laying eggs on substrate with this pesticide. They also can detect the presence of *Trichoderma* and will lay their eggs preferentially in areas contaminated with this fungus. The eggs are small, ¹⁄₁₆ inch long, translucent and white, and oval. They may be laid as singles or in large clumps. The larvae hatch from the eggs after about 4–6 days at regular compost temperatures (75–80°F, 24–27°C). The first instar larvae begin feeding immediately on mycelium and the compost itself. The larvae go through 4 instars to reach their maximum size of ¼ inch, shedding their integument at each molt to get larger. This is a vulnerable stage in the life cycle, and some insect growth regulators are active only on molting larvae. The larvae are voracious feeders and attempt to eat anything they find in their jaws as they move through the compost and casing. This includes other sciarid larvae (they are cannibals) and other insect larvae they might encounter in the compost and casing. They prefer to feed on developing mycelium and compost as opposed to a dense mycelial mat. It is hard for the larvae to feed on mycelium in fully spawn-run compost, as it is water repellant and studded with calcium oxalate crystals.

About 21 days after the eggs were laid, the larvae transform into pupae, the transition between the larvae and the adult. This stage is inactive and does not feed. Many times, the larva will spin a silk chamber to protect itself during pupation. Pupation generally lasts about a week. The males typically emerge 1–2 days before their sisters. Because there is a narrow window for oviposition during cooldown and spawning, the first generation of *L. mali* emerges as adults very synchronously just before first break. This synchronous development allows the grower to apply insect growth regulators and biological controls such as nematodes to the most susceptible life stages of the insect by timing development from the peak invasion on light traps. The complete life cycle requires about 28 days at normal compost temperatures. A peak of emergence usually can be seen for the second generation, but it is less distinct.

There is evidence that the timing of the life cycle for *L. mali* may change with development on different strains of *Agaricus bisporus,* different species of *Agaricus* (e.g. *A. blazei* or *A. bitorquis),* or different species of mushroom *(Pleurotus* or *Lentinula).* There have been reports that *L. mali* populations that have become resistant to certain pesticides may take longer to complete development.

The feeding of larvae in the first generation probably does little damage to the crop. The exception to this rule would be in situations where *Trichoderma* green mold is prevalent. In this case, it is likely that even small infestations of flies can significantly magnify the damage from this disease. Very high numbers of larvae feeding in the compost during spawn run also can inhibit fruit body production through destruction of the compost and the mycelium. In most situations, crop damage and loss of yield and quality result from the ability of the adults to mechanically spread mushroom diseases such as *Trichoderma, Verticillium fungicola,* and *Pseudomonas tolaasii.* The feeding of the second generation larvae also can be extensive and can result in yield loss through degradation of the compost and casing, and destruction of mycelium and fruit body primordia in the casing. In severe infestations, larvae can tunnel up into the stipe, resulting in the condition referred to as "black stem," which renders the mushrooms unmarketable. The potential for crop damage through reduced yield and quality is significant with this pest. Growers must be continuously vigilant to avoid crop damage from this insect pest.

Phorid Flies

A pest of secondary importance in North America is the phorid fly, *Megaselia halterata*. These flies are small, $\frac{1}{8}$ inch (2–3 mm) in length, with a humpback appearance and very small antennae (Figure 18). They appear stockier than sciarids and are very active, running and hopping erratically. The males and females closely resemble each other. Adult phorids typically enter the production rooms and houses later in the crop cycle than sciarids. They prefer warmer air temperatures and drier conditions in the substrate. They also can become a problem later in the year, typically June and July. Consequently, infestations of *M. halterata* are typically seen in drier areas of casing after second break. The larvae are creamy-white maggots that are no longer than $\frac{1}{4}$ inch (6 mm) when fully grown. The rear end is blunt and contains the opening of the breathing tubes. The head is pointed and the same color as the rest of the body. The mouthparts are relatively small hooks held inside the head. Phorid larvae feed only on mycelium and graze selectively.

Female phorids enter the growing room and lay about 50 eggs in areas where there is fresh mycelia growth. The larvae hatch after several days and begin feeding. They pass through three to four instars. They are more sensitive to variations in compost and casing temperature than sciarids, and the timing of the life cycle is variable. At warm compost temperatures of 75–80°F (24–27°C), development from egg to adult may require only 15 days. During cropping with lower temperatures (60–70°F, 16–21°C) in the casing, development may extend up to 50 days. The larvae feed only for about $\frac{1}{3}$ of this period of immature development. The remainder of the time is spent as the immobile and nonfeeding pupa. The pupae are about $\frac{1}{8}$ inch long and gradually turn from cream colored to dark brown as they mature. The pupae are flattened and oval in shape with breathing horns at the broad head end.

Figure 18. Phorid fly and larva.

Because the larvae feed selectively, they are not capable of causing the kind of damage that sciarids do as larvae. Significantly more phorid larvae can be tolerated—perhaps as much as 50 to 100 times more than sciarids—before economic damage can occur to the crop. Phorid adults are very capable of transmitting fungal and bacterial diseases, however, and control of the adults is necessary to maintain crop health. Because they are active fliers, they can be a significant irritant to picking crews, and control of the adults may be necessary to maintain efficiency.

Cecid Flies

A variety of other species of flies can be encountered in mushroom houses. The most potentially damaging are cecid flies (Figure 19). Three species have been identified as pest species in the United States. These three species are rarely seen as adult flies, because under most conditions larvae become "mother larvae" that give birth directly to 10–30 daughter larvae. These species usually do not become a pupa and subsequent adult that must mate before laying eggs. Reproduction is accomplished without mating and gives rise to daughter larvae directly. This is termed paedogenic parthenogenesis. When conditions are optimal, this method of reproduction can result in very rapid multiplication of this pest, leading to astronomical numbers of larvae, tens of thousands per square foot.

Cecid larvae are legless maggots, bluntly pointed at both ends. The head and the tail are not easily distinguished, except by the direction of travel. White larvae typically are the species *Heteropeza pygmaea,* while orange larvae are in the genus *Mycophila,* either *speyeri* or *barnesi. Heteropeza pygmaea* is probably the most commonly encountered cecid in mushrooms and has been reported from *Agaricus* as well as other species, particularly *Pleurotus*. The small, sticky larvae are spread by workers and on tools and equipment. Initial entry to the growing room may be by transport of infested peat or substrate, movement with personnel, or through the rare flying adult. Small infestations may not be readily apparent at first. The larvae feed on the mycelium as well as on the stipe and gills of mature mushrooms. If large populations develop, the larvae may mass together on the floor and disperse in large groups. Larvae also can be found on mature mushroom caps packed for market. This species has the potential to significantly reduce yield when it becomes established on a farm.

The two orange *Mycophila* are not as common as the white *Heteropeza,* but can cause significant damage. They have a slightly shorter life cycle and therefore can develop damaging population levels rapidly. However, their orange color makes them more conspicuous, and growers typically notice them before large populations are attained.

Cecid larvae have the potential of feeding on mycelium within wooden structures inside growing rooms. Because the wood offers some insulation from the heat of cookouts, they may survive the high temperatures and infest the next crop. Direct treatment of wood with insecticides and fungicides may be necessary to reduce between-crop survivors if there are high populations of cecids on the farm.

Figure 19. Cecid fly and larvae.

Other Flies

A number of other species of flies may be noticed by alert growers, especially on light traps. Most of these are incidental and may indicate that certain conditions can be found in growing rooms that require attention. Occasionally, they indicate the presence of a new pest on the farm. When in doubt about the identity of insects found on the farm, do not hesitate to submit samples for identification.

Flies that resemble a large phorid with prominent red eyes are probably fruit flies in the genus *Drosophila.* The term "fruit fly" is a misnomer for this group, as the larvae all feed on fungi of one sort or another, in some cases on rotting fruit. In the wild, larvae of these flies can be found feeding on mature sporophores in great numbers. These larvae resemble small house fly maggots in that they have a pointed head with small mouth hooks and a blunt rear end with breathing tubes. If we think of sciarids and phorids as feeding on the early stages of mycelial growth in the life cycle of a fungus, we can think of *Drosophila* as feeding late in the life cycle. If you see significant numbers of *Drosophila* adults, there are probably areas in the growing room with large, over-mature mushrooms. This can be a problem particularly in portobello production if large, nonmarketable mushrooms are not picked off the bed promptly. The danger here is that if large populations develop, eggs may be laid on mushrooms packed for sale. If the eggs hatch and larvae begin feeding during transit, storage, and display in the retail store, consumers may purchase mushrooms with maggots in the cap.

Other species of flies may indicate that anaerobic conditions may have developed at some place on the farm or in growing rooms. These flies include black scavenger flies (sepsids), moth flies (psychodids), and small dung flies (spherocerids).

C. Pest Species Biology and Control

2. Fungal Pathogens

Phillip S. Coles
William Barber

Introduction

There are many fungal pathogens of mushrooms, but only a few of them currently affect commercial mushroom farms. Some of these are true pathogens attacking the mushroom mycelium, while others can simply outcompete mushroom mycelium growth. Fungal pathogens can either affect the quality of the product, reduce production, or both. But all of them reduce the total return of a crop, often significantly. Many control methods, such as sanitation, are useful for all of the diseases. There also are control measures specific to each disease.

Verticillium Diseases

Common names:
Verticillium disease, *Verticillium* spot, brown spot, fungus spot, dry bubble

Scientific name:
Verticillium fungicola

Outdated names:
Verticillium malthousei, Acrostalagmus fungicola, Cephalosporium constantini

Perfect stage:
unknown

Verticillium is one of the most significant diseases of commercial *Agaricus* production. It is endemic on many mushroom farms and can cause substantial yield reduction. It can occur in nature in addition to cycling within a mushroom farm, traveling from older to newer growing rooms.

Identification

Infection takes on a variety of forms and has various symptoms, from small spotting on the surface of a mushroom cap to a complete infection of the fruiting body so that it is unrecognizable as a mushroom. Appearance will depend on the timing of infection and the number of spores.

The first symptom group is spotting, a superficial infection causing necrotic lesions on the cap of the mushroom. These spots will enlarge and coalesce as the mushroom enlarges. This easily can be confused with bacterial blotch or *Trichoderma* spot. Spotting is the result of late infections of *Verticillium*. The mushroom already had developed when the infection occurred, and the pathogen only had time to infect the mushroom superficially.

A simple way to determine which organism is causing spotting is to place infected mushrooms into a sealed plastic container with a few moistened paper towels. The water in the towels will keep the humidity of the chamber high, and the causal agent will grow out from the mushroom tissue. If the infection is bacterial, the color of the spots will not change. *Trichoderma* spot, on the other hand, will turn green when the fungus sporulates, and *Verticillium* will turn the mushroom surface gray and give it a fuzzy texture.

A very localized infection on the mushroom cap can be expressed as a "harelip." The infection kills the cells in a specific area, preventing growth. Then, while the other cells of the cap continue to grow, expansion occurs everywhere except within the infected area. This causes the pinched area, or harelip. The dead area of the sporocarp will appear gray and leathery.

Infections on the mushroom stem will cause exterior cells to die. Because the exterior cells no longer will grow while the noninfected cells continue to elongate, the mushroom will bend towards its infected side. Further, the dead cells will split and crack, causing a "blow out" (stipe blast) on the side of the mushroom stem. An infection on the stem also can be expressed as a streak along the length of the stem.

More significant infections cause serious deformation of the sporocarps, which will appear as large, formless, puffball-like masses. The cap becomes indistinguishable from the stem (Figure 20). Growers commonly refer to this symptom as "dry bubble." Its expression requires early infection by *Verticillium* spores. The *Verticillium* spores must have infected the pins early enough, and with enough spores, to have time to completely take over the growth of the pin. Bubbles will be covered with the gray fuzzy bloom of the *Verticillium* conidiophores.

Figure 20. *Verticillium* in the dry bubble stage.

Figure 21. *Verticillium* spot.

Biology

Verticillium spot (Figure 21) takes about 7 days to produce visual symptoms. If there are visible lesions, stipe blast, or other superficial mushroom deformities, it can be concluded that *Verticillium* spores infected that mushroom about 7 days before the appearance of symptoms. For an actual bubble to appear, the infection requires a 10- to 14-day incubation period. Therefore, if the mushroom pin already is formed at the time of infection, there will be only superficial markings on the mushroom. If the infection begins soon after casing, dry bubbles will be formed.

Verticillium needs the developing sporophore to manifest symptoms. The mycelium germinating from the *Verticillium* spores will grow into the mushroom tissue, parasitizing and deforming it. Mushroom mycelium alone will show no symptoms of *Verticillium.*

Verticillium infections are caused by spores and mycelium transported or spread to uninfected sites by many different modes. The spores are very sticky and can be carried by anything to which they are able to stick. This includes, but is not limited to, personnel and their clothing, mushroom flies, mites, and rodents. Flies are particularly problematic vectors, since they actively are trying to leave older growing rooms. Flies likely will pick up *Verticillium* spores in the older rooms and spread the infection to new rooms. In addition, they can carry mites that in turn can transport *Verticillium* spores.

Once the mites leave the bodies of the flies, they will spread spores while moving throughout the room. Rodent fur is an excellent carrier for the sticky spores, and the tendency for mice and rats to bore into mushroom beds in search of spawn grains can expose a lot of material to infection. Equipment can be a source of inoculum, especially equipment that is moved from dirty areas to clean areas. A good example is watering or spraying equipment. Watering and spraying are done throughout the crop, and if a watering nozzle or hose is used at the end of a crop and then moved to an earlier stage, infection can result. Harvesting baskets traveling to and from a processor also can be a source of inoculum if the baskets are delivered from an infected growing area to the processing plant and are returned to the farm—or delivered to another farm—where they might infect a previously clean growing area.

Verticillium can be spread on air currents. Spores will stick to dust particles and can enter a growing room through the ventilation systems. Dust can settle on equipment or casing materials en route to a room. Spores also can travel on airborne mites, regardless of whether or not the mites are living.

The initial infection may come from one of many sources, but once inside a room, the infection can spread very quickly. This is due to the high reproductive capability of the *Verticillium* organism, which can produce 30 million spores per hour. Tests on petri plates have shown that, after touching one bubble that is sporulating, a finger can touch eight more petri plates and cause infections on every plate. Therefore, anything contacting a sporulating bubble can infect many potential sites. High fly populations are very effective at spreading an infection throughout a room. Water hitting an infected site can pick up spores and splash them onto other mushrooms, infecting them with *Verticillium*. Harvesters and their equipment will spread an infection quickly throughout a growing room, as well as from room to room.

High spore loads can develop on the floors and other infection sites, increasing the possibility of spores being picked up by a vectoring agent. Dead mushrooms also can be reservoirs for inoculum.

Monitoring

The most useful method of monitoring *Verticillium* is to count the number of bubbles in a growing room. By mapping the number and location of the bubbles, you can detect patterns. Improperly sanitized casing equipment may show itself in a high concentration of bubbles in the area where the casing crew starts. High bubble counts in rooms having the highest incoming fly populations could indicate spores coming in with flies. High bubble populations near doors might suggest that dust is entering through doorways or that there are possible ventilation problems.

Understanding the timing of *Verticillium* disease is essential for controlling it. The time when a specific symptom manifests itself is a good indication of when the infection occurred. If bubbles appear on first break, for instance, there probably was a breakdown in sanitation in the peat moss preparation, the casing operation, or an early stage of case growing, since there is a 10- to 14-day incubation period for bubble development. If bubbles do not occur until the last break, it is likely that spores are entering once harvesting has begun, either on harvesters or harvesting equipment.

Control

Verticillium control depends primarily on eliminating spores through sanitation and control of vectoring agents. All equipment should be kept in dedicated storage areas. Equipment and personnel from dirty areas never should be allowed to enter clean areas, and personnel and equipment from clean areas never should be allowed into dirty areas. If hoses or spray apparatus, for example, must be moved between clean and dirty areas, they should be moved from newest to oldest rooms, then sanitized before they are returned to new rooms. (See "Sanitation" in Chapter II.A.2, Cultural Control.)

Harvesters must be trained to recognize and not touch bubbles. More importantly, employees must be taught the importance of cleanliness, particularly if they work in clean areas. Control dust by paving roads or by oiling or watering gravel roads. Filter air to exclude spores and anything that may be carrying them, such as flies or mites. (See Chapter II.A.1, Exclusion.) Control fly and mite populations and their movements into new growing areas. (See Chapter II.C.1, Arthropod Pests.)

Bubbles can be destroyed with salt. The best method is to put salt into a plastic drinking cup, then cover the bubble with the cup and salt (Figure 22). The salt will desiccate the bubble, preventing further mycelium growth, and the plastic cup will prevent the spores from spreading. Bubbles can be physically removed from the growing room. This is often done in an alcohol solution. The procedure is risky, however, since the bubble is disturbed and spores might be released. Worse yet, the person removing the bubbles can become a disease vector.

Figure 22. Salt will kill the *Verticillium*, while the cup will prevent the spread of spores.

Fungicides have been, and most likely will continue to be, available for the control of *Verticillium*. There is, however, a special difficulty with trying to develop a fungicide for a fungal pathogen of a crop that is itself a fungus. Very often the fungicide will have a toxic effect on mushroom growth. This must be weighed against the benefit of *Verticillium* control, since some mushroom production could be lost. Also, since pesticides that are the least deleterious to the mushroom crop must be used against *Verticillium*, the fungicide's mode of action against dry bubble must be targeted to one of the few things that *is* different about *Verticillium* and *Agaricus*. Since mushrooms and *Verticillium* are very similar from a pesticide's point of view, any differences between the two that are exploited by a pesticide's mode of action would be small, and there would be a stronger propensity toward the development of resistance than normally occurs in most pest species. Therefore, pesticides should be used sparingly, only when needed, and according to economic thresholds (See Section I.B).

In some instances, despite whatever combination of control measures are used, *Verticillium* can run rampant throughout a growing room. Sometimes it is possible for every developing sporophore to be expressed as a bubble. In this extreme example, there is no point in continuing the crop, especially if no harvestable mushrooms are being produced. The room will have become an incubator for *Verticillium* spores and most likely will be producing flies that will further spread the spores and those of other molds. Trying to save old crops with this level of infestation will result in the continuation of the infection cycle. Steam the room early and eliminate this potential source of inoculum.

Trichoderma Green Mold

> **Common name:**
> green mold
>
> **Scientific name:**
> *Trichoderma harzianum*
>
> **Perfect stage:**
> unknown

Trichoderma harzianum is a relatively new disease of commercial mushroom production. It was first encountered in Ireland and the UK in 1985. During the 1985–1986 growing season, the ensuing epidemic caused losses estimated at one million monetary pounds ($l.5 million U.S.). Through 1990, losses were estimated to be between 3 and 4 million pounds ($4.5–6.0 million U.S.). In 1990, it appeared in British Columbia, and in the Ontario area in 1992. In 1993, it reached the Berks County growing area of Pennsylvania and, in 1994, Chester County, Pennsylvania. Since then, it has become endemic in Pennsylvania.

Aggressive strains of *Trichoderma harzianum* have been associated with the commercial production of *Agaricus bisporus*. In the UK, the aggressive form is known as "Th2." In the U.S. and Canada, "Th4" is the dominant aggressive strain. These aggressive strains have been found only on mushroom farms and only recently.

The genus *Trichoderma* includes many common soil-inhabiting fungi and decaying organisms associated with wood and decaying vegetation. In nature, it has an important role as a decomposer. *Trichoderma* is a very complex genus, and not until 1969 did Rafi properly clarify the taxonomy. Nine species aggregates were identified

from their microscopic characteristics, but to date there is still no satisfactory classification of species in *Trichoderma*. In addition, there are many different strains or races in the various species. They can vary in aggressiveness, resistance to heat or pesticides, and in a variety of other ways.

Trichoderma species are asexual fungi that propagate through vegetative growth and production of asexual spores (conidia). The conidia are spread easily by various means. *Trichoderma* also can have a sexual stage in which its appearance is changed so substantially that it originally was classified incorrectly as belonging to the genus *Hypocrea.*

The members of the genus *Trichoderma* have a considerable arsenal of "chemical weapons" that are produced in the form of antibiotics and other toxins that strongly inhibit the growth of other organisms. Furthermore, some species are capable of parasiticism on the mycelium of other fungi. Its aggressiveness makes it useful as a biological control agent against fungal pathogens of green plants. This same aggressiveness, however, makes it a serious pathogen in commercial mushroom production.

It is now possible to isolate different species and strains through Polymerase Chain Reaction (PCR), but when first encountered, green mold samples had to be identified through microscopic examination that was very time-consuming and always suspect as to accuracy. PCR examination also has shown that green mold is not a new strain of *Trichoderma* that mutated from an existing form, nor is it one of many strains developed for biological controls on green plants. It probably has been around for millions of years, and changes in cultural practices made it very successful in mushroom houses.

Beyond mushroom farming, it is very rare.

Trichoderma mycelium grows on compost and competes aggressively with mushroom mycelium. Microscopic observation of the interaction between *Trichoderma* and mushroom mycelium does not show any obvious pathogenicity. This has lead to debate about whether *Trichoderma* green mold is a fungal pathogen or a competitor.

Identification

Trichoderma mycelium is gray in the beginning and then changes to white, becoming very dense. After fruiting, its spores turn it a dark green (Figure 23). There are many other types of molds that also are green and associated with mushroom compost, including *Gliocladium, Cladosorium, Asperigillus, Penicilium,* and *Chaetonium.* Care must be taken not to confuse them with green mold. There also are other species and varieties of *Trichoderma* that will not cause the disease, and only through close taxonomic examination or through PCR can they be differentiated. However, if green mold progresses rapidly across the growing surface (Figure 24), it can be assumed to be one of the aggressive varieties of *Trichoderma* green mold.

Pygmy mites often are associated with green mold infestations, though this is not always the case. They also can occur in the presence of other types of fungi.

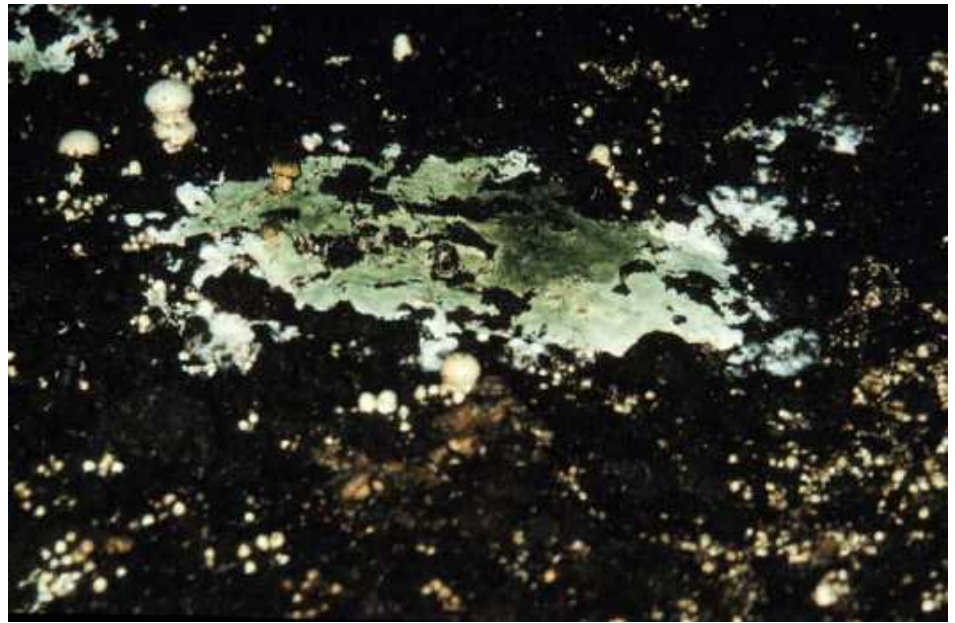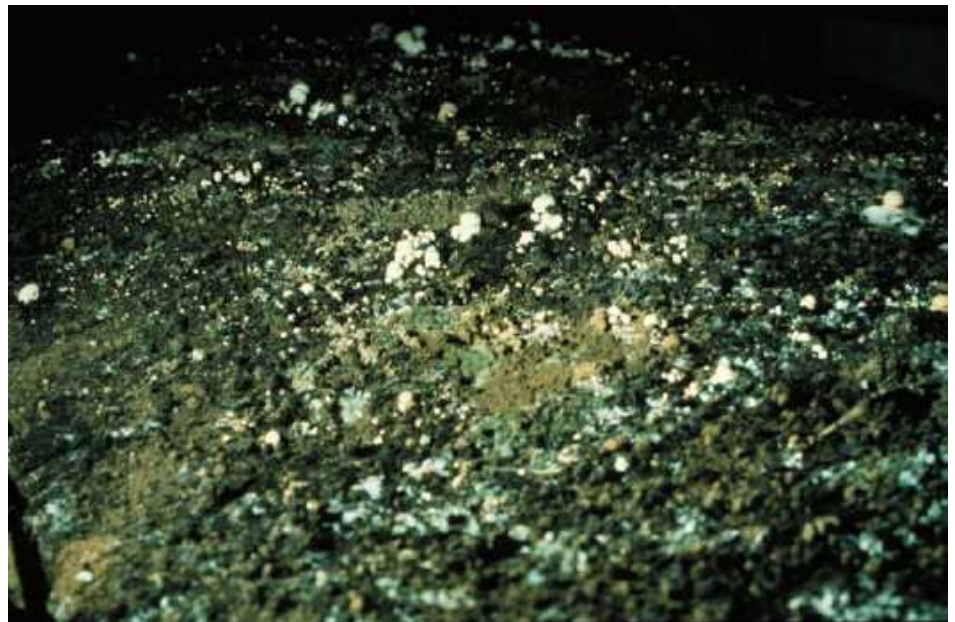**Figure 23. *Trichoderma* mycelium, showing dark green color.**

Figure 24. Widespread green mold infestation.

Biology

To infest a mushroom crop, *Trichoderma* first must have its spores introduced. The spores are contained in a sticky matrix that can attach to many different surfaces. Consequently, many of the traditional pathways of other types of fungi also apply to green mold. The spores can adhere themselves to employees and their clothing, as well as to equipment used on the mushroom farm. Rodents can carry spores, and spores can travel on flies or on mites carried by flies. (Mites are excellent vectors because they have specialized organs known as sporangia, which are used to spread fungal spores.) Mushroom trimmings can be a reservoir for spores, and the practice of putting trimmings in compost can add to inoculum sources. If post-harvest and Phase II pasteurization are insufficient, green mold spores can survive to infest a new crop.

It is not enough for spores simply to be present; they must exist in sufficient numbers and correct conditions must prevail. No specific compost or environmental conditions have been found to be associated consistently with green mold development. It has been shown that a carbohydrate source is necessary for spore germination. Spawn grains serve the carbohydrate requirement very well if they are fresh (the mycelium has not yet grown into the compost) and if the green mold spores are within one centimeter of the grain. Green mold, therefore, will not germinate in fully colonized compost, where the mushroom mycelium protects the grain from the disease. Green mold spores introduced at casing will not germinate for the same reason. Also, a minimum number of spores are required. Theoretically, only one spore is needed to start a green mold infection; but, as is true with most types of fungi, one spore is not enough. Grogan showed that it is possible to get an infection from less than 100 spores, though normally more are needed. For experimental purposes, at least 9 million spores are used in each inoculation.

Once germinated, the green mold mycelium will move quickly into compost and colonize it. Consequently, mushroom mycelium no longer will be able to grow there. The green mold then will move into compost already colonized by mushroom mycelium and will spread across an entire growing surface.

Monitoring

Use the *Verticillium* mapping technique to monitor green mold; i.e., count the number of squares infected with green mold in a growing room and map the number and location of the infections. By noting the number and location, you can detect patterns. Improperly sanitized spawning equipment may show itself if the highest concentration of green mold is in the area where the spawning crew starts. High green mold counts in rooms or in areas of a room having the highest incoming fly populations could indicate that spores are coming in with flies. High green mold populations near doors could indicate that dust is entering through doorways or that there are possible ventilation problems.

The time at which a specific symptom manifests itself is a good indication of how severe the infections were at spawning. If no green mold is detected except for a few spots at the end of the crop, the amount of inoculum probably was low. If it is seen when the plastic is pulled at spawn run, there was a serious infestation.

Control

Control begins in Phase I and Phase II composting, where the number of spores in the compost must be minimized. Any green mold spores that may get into the compost during these stages must be destroyed to prevent germination in the growing rooms after the room is planted.

Minimize potential inoculum sources by not allowing unpasteurized materials from harvesting, such as mushroom trimmings, onto the compost wharf where green mold spores could collect in the leachate pond. It is better to remove all trimmings from the farm site if possible.

To eradicate spores that may get into the compost, cross-mix during Phase I so that all the material is exposed to the highest composting temperatures possible. Control moisture to ensure that the maximum amount of compost reaches these temperatures. Formulate so there is a distinct ammonia odor at the end of Phase I. The ammonia will help to degrade the exterior of the spore coat.

Phase II pasteurization must be complete. Pasteurize at 140°F (60°C) for two hours. Beds must be filled uniformly to ensure that all areas attain this temperature.

Disease control depends primarily on eliminating spores through sanitation and control of vectoring agents. Sanitation at spawning is more important to control of green mold than, for instance, control of *Verticillium*, which is more dependent on control after casing. Harvesting and overall farm sanitation are important for control of both organisms. All equipment should be kept in dedicated storage areas. Equipment and personnel from dirty areas never should be allowed to enter

clean areas, and personnel and equipment from clean areas never should be allowed into dirty areas. If hoses or spray apparatus, for example, must be moved between clean and dirty areas, they should be moved from newest to oldest rooms, then sanitized before being returned to new rooms. (See more on sanitation in Chapter II.A.2.) Control dust by paving roads or oiling or watering gravel roads. Filter air to exclude spores and anything that may be carrying them such as flies or mites. (See more on exclusion in Chapter II.A.1.) To further reduce green mold spore spread, take the additional step of using separate cafeterias and break rooms for employees working in areas other than harvesting. This also will help with other types of pathogens. Employees working in the spawning area should be issued new uniforms daily.

Areas infested with green mold can be controlled with salt or hydrated lime by sprinkling the infected areas with either material. The salt or lime should extend at least 8 inches from the edge of the visible growth, since adjoining casing can harbor the mycelium and soon be producing spores. If mycelium appears on the surface of the compost before casing, spray the area with a 1,000-ppm chlorine spray, again extending the treatment 8 inches beyond the infection's visible edge.

Existing chemical pesticides essentially are ineffective on green mold mycelium once it is growing actively in compost or casing. However, it has been shown that some types of fungicides applied to spawn grains will provide limited protection to the grains and prevent green mold spores from germinating. Tumbling the spawn with the fungicide mixed with a carrier such as gypsum coats the grains. Great care must be taken when applying fungicides to the

spawn. First, spawn must be kept clean. In order to be mixed with a fungicide, spawn must be removed from its original packaging, exposing it to possible contamination. This could result in a worse green mold infection than if no fungicide had been applied. Therefore, sanitation is of the utmost importance during this operation.

Second, tumbling the spawn in a mixer damages some of the mushroom mycelium on the spawn grains and can reduce its vigor. Mix the grains as gently as possible. Once it is mixed with the fungicide, spawn usually is stored in a larger bag than the original packaging. Therefore, care must be taken to prevent overheating of the spawn before it is applied to the compost. Of course, pesticide labels must be followed at all times.

Fungicide applications should be limited to avoid resistance development. They should be used as a "last resort" when green mold infestation is out of control. Sanitation is the only way to control green mold in the long term, and once the disease is brought under control, the use of fungicides *must* be stopped to reduce the chance of development of resistant green mold strains. (See more on resistance management in Section I.A and Chapter II.A.4.)

As with *Verticillium*, if green mold gets out of control, it is better to steam off the room early rather than risk the spread of infection to new rooms. Reducing the amount of inoculum will yield benefits to future crops that are greater than the benefits that might be reaped from the few mushrooms that could be salvaged. Post-harvest must be adequate.

Dactylium Diseases

Common Names:
cobweb mold, *Dactylium* mildew, soft mildew, soft decay

Scientific Name:
Dactylium, cladobotryum

Outdated names:
Dactylium dendroides, Nectria albertinii, Nectria rosella, Cladobotyium dendroides

Identification

Dactylium mildew, or cobweb mold, can be recognized by its wefty, cotton-like mycelium. The mycelium will cover the surface of the casing as well as the surface of mushrooms and mushroom pins. The mycelium is usually white, but can be gray and often turns pink or yellow with age. Infected mushrooms develop a soft, wet rot.

Cobweb mold is a relatively minor disease of mushrooms, but because of its ability to grow quickly, it can spread over many mushrooms. If left unchecked, widespread mildew can result in unsalable mushrooms and eventual significant yield loss.

Biology

Cobweb mold occurs only on the casing layer and cannot grow in the compost. Therefore, infection must take place after casing. Symptoms can occur before first break, but they usually appear later in the crop. *Dactylium* may thrive in the controlled environment of a mushroom facility, but it also can survive in wild mushrooms or in soil. Inoculum can come from outside sources surrounding a mushroom farm or from older rooms where infections have occurred. Unpas-

teurized soil or spent mushroom substrate used for casing can be a source of inoculum. Actually, any type of casing can cause infection if it has become contaminated. Further, spores can enter a growing room through ventilation or on employees or equipment. Infection often begins on dead material left on the casing surface. Dead fruiting bodies or the stumps trimmed from mushrooms can be a food source for germinating spores. From there, the infection can spread to the casing layer, covering it and any mushrooms or pins in its path. Infections can appear quickly and can spread rapidly. Trash left on beds, high relative humidity, and high air temperatures are very conducive to cobweb mold's growth.

Control

Cultural controls, especially sanitation and exclusion, are the best way to control cobweb mold. Casing areas must be kept clean and sanitized. Casing material must be loaded into sanitized trucks and covered to prevent contamination during transport to growing rooms. All equipment used for casing must be cleaned and sanitized. Casing employees must be clean and wearing laundered clothing each day. Once the casing material is safely inside the room, the air must be filtered to ensure cobweb spores do not enter the room (see Chapter II.A.1, Exclusion). Beds must be kept clear of trash such as stumps or dead mushrooms, where infections can start.

Environmental control is the key to preventing the spread of existing infections, since cobweb mold needs both high humidity and high temperatures to spread. Often, growers will raise a growing room's temperature to accelerate mushroom growth. A grower may be trying to outpace the growth of a pathogen or trying to complete a break on schedule. This practice can cause more harm than good, for, if it is done when cobweb mold is present, an epidemic may occur because the mold's rate of growth will increase faster than that of the mushrooms. Maintaining the optimal temperature for mushroom growth, on the other hand, will be detrimental to the growth of cobweb mold.

Since high humidity promotes the growth of cobweb mold, it is very susceptible to control by desiccation if growing room relative humidity is lowered. Maintain the room temperature below 65°F (18°C) and the relative humidity below 92 percent, and the growth of cobweb mold will be inhibited.

Chemicals also may be used to control cobweb mold, though presently there are no materials registered specifically for it. Some fungicides applied for other types of pathogens such as *Verticillium* have the unintended but beneficial effect of controlling cobweb mold.

C. Pest Species Biology and Control

3. Weed and Indicator Molds

David M. Beyer

Introduction

Weed molds may be defined as molds that grow in competition or in association with the mushroom mycelium. These fungi compete for nutrients and may have a negative influence on the growth and nutrient uptake by *Agaricus bisporus;* however, they are not known pathogens. Some weed molds may grow in properly prepared compost for supporting the mushroom's growth, while others may not grow unless the mushroom mycelium is present. The range of effect that weed molds may have on the mushroom mycelium is broad.

Indicator mold are fungi that grow in compost that has not been selectively prepared for *A. bisporus*. Growth of these molds may suggest a nutritional imbalance in the compost. Indicator molds will grow only in compost that has specific nutrient conditions that favor their development. These molds grow on compounds that the mushroom cannot use, and once that food source is depleted, these molds will stop growing and usually disappear. However, because compounds were available to these fungi, fewer nutrients are available to *A. bisporus,* and crop yield usually is lowered.

Some of each type of mold have little to no effect on *A. bisporus*, while others can entirely inhibit the growth of the spawn and eventually the mushrooms.

Weed Molds

Lipstick Mold

Common Names:
lipstick, red lipstick

Scientific Name:
Sporendonema purptirescens

Outdated Name:
Geotrichuin candidutti, Oosporum sp.

Lipstick mold may occur in compost during spawn run or in the casing during cropping. At first, this mold is hard to distinguish from spawn growth, as it first appears in spawned compost as a white crystalline-like mold. Growth begins as small white colonies, previously referred to as "frost on a windshield" or "small white cotton balls" on straws or casing. When developing after casing, these small white balls may be misidentified as mushroom spawn forming into pins. The descriptive lipstick color develops as the spores are maturing. Several shades of pink, cherry red, and eventually orange or buff colors may be found (Figure 25). It has been reported that lipstick in a peat moss and limestone casing remains white, and its red color will not develop.

Figure 25. Lipstick mold causes a descriptive lipstick color as the spores are maturing. Several shades of pink, cherry red, and eventually orange or buff colors may appear.
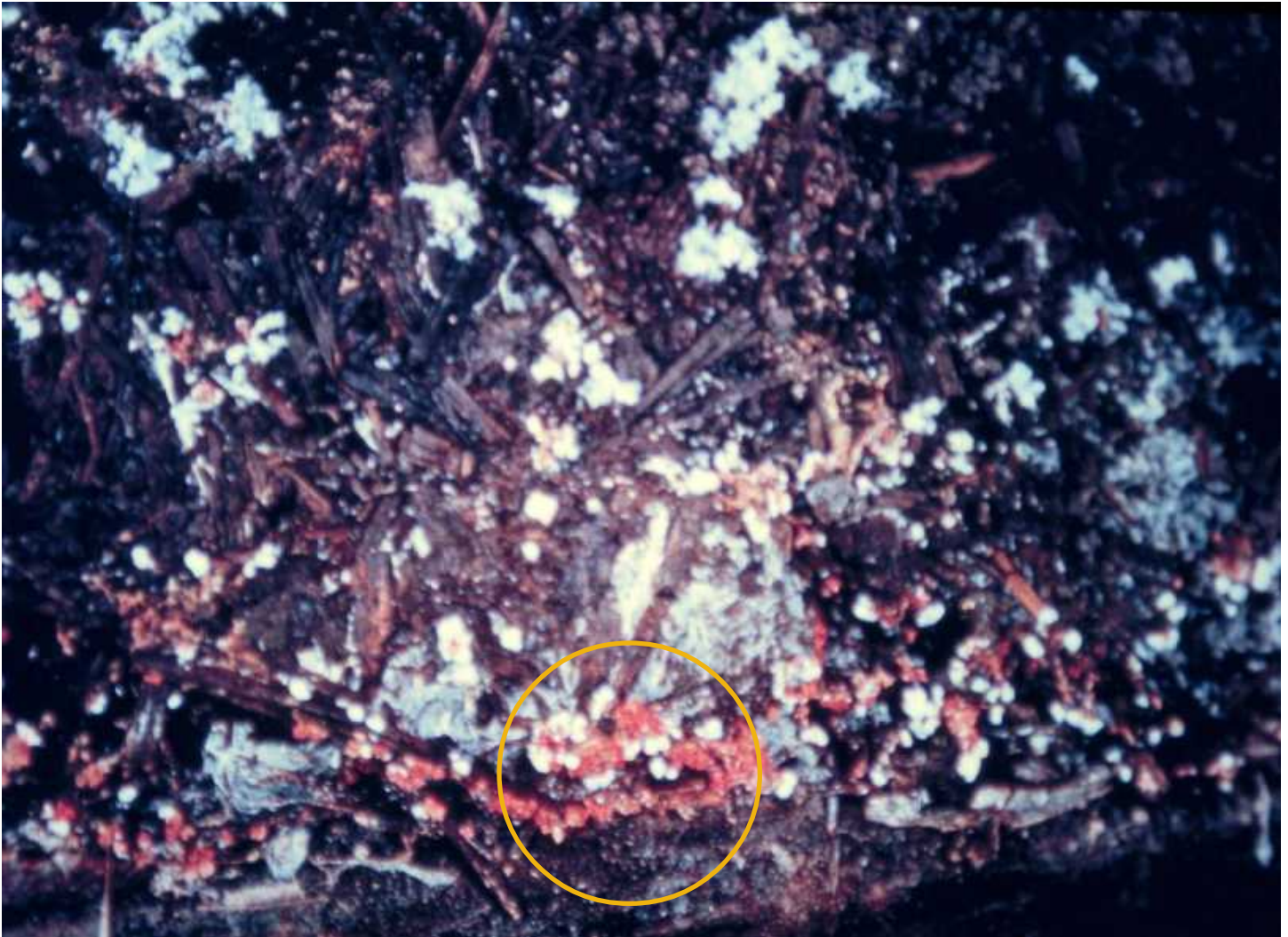
Lipstick mold grows slowly and usually remains confined to areas of the compost or casing. It does not appear to grow outward like green mold or mildew. The white growth of lipstick eventually may grow into uninfected areas of casing, and it is able to colonize well-conditioned compost. Significant yield losses are associated with heavy compost infestations prior to casing. If the mold does not become visible until third break, yield loss will be minimal.

Air currents can spread spores from contaminated casing or spent compost during watering or via pickers. Heavy infestations usually reflect a build-up of spores around a mushroom production area. Poor sanitation and inadequate post-crop steaming are possible causes for an increase in spores around a facility.

An infestation of lipstick mold may continue for several crops or cycles on a farm. Control is achieved through a complete post-crop steaming and adequate pasteurization during Phase II. The lipstick fungus may not be a proven pathogen of the mushroom, but its presence indicates the need for increased sanitation and pasteurization procedures.

It has been reported that the occurrence of lipstick mold would indicate that a La France virus disease might also be present. However, when virus occurs, lipstick mold is not always present. This phenomenon suggests that the virus-infected and lipstick spores are spread around or introduced into an area in a similar manner. Some of the control methods for this mold would be similar to those for LaFrance disease.

It also has been suggested that the occurrence of lipstick is related to old wet poultry manure; wet, dense compost at filling time; or excessive use of steam during Phase II. In addition,

excessive nitrogen at spawning time may be related to increased lipstick mold. Excess nitrogen may be a result of the wet compost or excessive moisture condensation with too much steam. In these latter cases, other molds also may be present with lipstick. Wet compost or lumps of wet chicken manure may not be completely pasteurized, and lipstick spores may survive.

Cinnamon Brown Mold

Common Names:
brown mold, cinnamon brown mold

Scientific Names:
Chromelosporium fulva, Chromelosporium ollare

Outdated Names:
Botrytis crystalline, Ostrachoderma peziza

Perfect Stage:
Peziza ostrachoderma (cup-shaped fruiting bodies)

Cinnamon brown mold has a variety of color ranges, from yellow gold to golden brown to cinnamon brown. Cinnamon brown mold is one of the most common brown molds found in mushroom houses. The mold first appears as large circular patches of white or gray-white aerial mycelium on the compost, casing, or on bed or tray boards. This mold may grow on compost, but it is most frequently seen after casing. The mold starts out white, but within a few days spores form and the color changes to light yellow or to light golden brown (Figure 26). Over time, the color deepens to golden brown or cinnamon, and the mold develops a granular appearance. As the center of the mold

colony becomes cinnamon-yellow brown, the edges will remain white. The mold grows rapidly but usually disappears within 10 days or by the time mushrooms are first harvested. It is possible that a dense infestation will retard the crop, especially first break, and cause a slight yield reduction.

The fungus, *Chromelosporium fulva,* is extremely common in soil and flourishes on damp wood. Under certain conditions, it can grow into casing not colonized by spawn. Areas in compost that overheated during spawn run, virus- or *Trichoderma harzianum*-infected areas, or areas of wet compost at fill with poor spawn growth encour-

Figure 26. Cinnamon brown mold starts out white, but changes color to light yellow or golden brown.

age the growth of cinnamon brown mold. This mold has been observed growing on undistributed supplements added at spawning. Improperly conditioned compost containing green mold often will contain cinnamon brown mold. Widespread infestations of cinnamon brown mold may indicate either poor sanitation or wet and improperly conditioned compost.

The mold is most commonly known as a re-colonizer of over-pasteurized casing and spent compost. The mold will grow rapidly from infested compost areas into casing, especially in areas where spawn growth is weak or nonexistent. It will grow on the casing and can become obvious throughout much of the growing room at the same time, suggesting that airborne spores landed on the casing at about the same time. The high humidity and warm temperatures following casing are ideal for growth of cinnamon brown mold.

Several weeks after first appearance of the mold, and after the mold has disappeared, small cups or disk-shaped fruiting structures may appear on the casing; these are the sexual phases of the *C. fulva (Peziza ostrachoderma)*. The cup-shaped structures have a rubbery or leathery texture and usually are dark brown, although chartreuse and yellow fruiting bodies have been observed (Figure 27).

Figure 27. The cup-shaped structures caused by cinnamon brown mold have a rubbery or leathery texture and usually are dark brown, although chartreuse and yellow fruiting bodies have been observed.

Sepedonium Yellow Mold

Common Name:
yellow mold

Scientific Names:
Sepedonium spp., *Sepedonium chrysosporium*

Sepedonium yellow mold begins to grow as a whitish mold that eventually turns yellow with age, and produces abundant spores that become easily airborne. Yellow mold differs from other yellow-colored molds by the appearance of thin white mold growing in compost during the spawn run and by the tremendous spore load that develops. The spore load causes clouds of "dust" when compost is disturbed (Figure 28). The sparse white mold turns dull yellow to tan with age. Yellow mold spores can be spread to compost by air currents before or during the filling operation, during the spawning operation or spawn-running period, or because of spent compost sticking to wooden boards or trays. Spores also may survive pasteurization in compost that is not conducive to good heat conduction and does not reach adequate temperatures.

The obvious, thick-walled spores of *Sepedonium* are resistant to the high heat of pasteurization; therefore, they are able to survive Phase II. These spores are spherical, golden brown, large, and distinctly spiny, a characteristic that distinguishes *Sepedonium* from the other significant compost yellow mold, *Chrysosporium*. The latter causes mat and confetti diseases. *Sepedonium* produces smaller oval spores, but these are rarely observed in mushroom compost specimens.

The growth of *Sepedonium* seems to affect spawn growth—the mold colonizes compost considered ideal for spawn growth. Heavy infestations of *Sepedonium* yellow mold are associated with poor yields, but whether this is due to *Sepedonium* or to other factors is not known. *Sepedonium* spore populations will build up on a farm following the appearance of yellow mold. Strict temperature monitoring and control during compost pasteurization and an adequate post-crop pasteurization are essential to eliminate the threat of infestation. Preventing spores from entering mushroom houses during spawning and the spawn-running period is essential. High-efficiency air filters reduce the possibility of introducing the mold into spawning areas, and sanitary conditions should be maintained during spawning.

Figure 28. Yellow mold has a distinct yellow color in compost. The tremendous spore load of yellow mold causes clouds of "dust" when compost is disturbed.

Pythium Disease

Pythium is an antagonistic, potentially pathogenic fungus infrequently isolated from mushroom compost. The fungus has the potential to cause yield loss, because spawn will not grow in areas colonized by *Pythium.*

Toward the end of spawn run, perfectly round areas may be noticed where spawn does not colonize the compost. These distinct circular areas, which may vary in size from a few inches up to 1–2 feet in diameter, are characteristic of compost infested with *Pythium*. The compost immediately adjacent to these black areas may be well colonized with spawn and support a normal crop of healthy mushrooms. Occasionally, the compost surface may be grown over with spawn, but a lens or football-shaped mass of black compost, with the greatest diameter in the center, may be found by digging into the compost. At the compost's surface, only a small (2- to 3-inch) black spot may be seen, but on digging into the compost, the characteristic shape would become apparent. The compost may contain no signs of a pest or pathogen except for the sparsely growing delicate white mold, which is *Pythium*. Eventually, spawn may colonize the infested compost; however, few if any mushrooms will grow in these areas.

Microscopic examination and laboratory study are necessary to identify a white compost mold and confirm the presence of *Pythium.* Often, other diseases or improper cultural practices cause spotty mushroom production.

Little information is available on the life history of this fungus and the mechanisms by which it spreads throughout a mushroom production area. Pythium spores are large and thick-walled, and may survive various heat and moisture treatments. It has been reported that they are resistant to heat and drought. Viable spores have been recovered from dry surface compost after Phase II, and spores can survive up to 18 months at room temperature. Severe *Pythium* development occurs after spores have been introduced to compost at or before spawning. Airborne spores that contaminate compost at spawning time are reported to be the primary source of infection. Therefore, filtration and reduced spore loads during Phase II and spawning will help to control this mold. Apparently, spores introduced a few days after spawning will not become established in compost and will not prevent spawn growth. Soil-laden straw or horse manure also are thought to be sources of spores that survive pasteurization and then colonize within compost. Control also is accomplished with sound cultural practices such as effective pasteurization of compost during Phase II, a comprehensive sanitation program for spawning, and a complete post-crop steaming.

Corticium Mold

Corticium mold is found in compost, on casing, or on the woodwork in growing rooms. This flat-growing gray-white mold is found on straws or wood in mushroom houses. It appears to grow from within beds or tray boards, uprights, cross pieces, and other wood structures. When the mold grows on casing, it looks granular like salt. Small 1- to 2-inch diameter circles, occasionally covering up to 65 percent of the casing, will be found. Corticium is found infrequently today because of effective pasteurization and post-crop steaming procedures. When this mold does appear, it may tend to persist for several consecutive crops until it is concurrently eliminated from infested wooden surfaces and compost.

Overly decomposed—but not necessarily wet—substrate is associated with the development and occurrence of corticium in compost. Widespread infestations will result in yield reductions of up to 10 to 20 percent, and reductions as high as 40 percent have been reported.

Corticium grows naturally as a common rotter of cellulose (dead tree limbs, stored straw, etc.) and profusely sporulates when the weather is damp. It is possible that spores of the Corticium-like fungi are carried by air currents into a mushroom house before or during the spawning operation, or whenever the growing room is opened to the outside environment. Improperly cured compost is a good substrate for this mold. Yield reductions can be attributed to either the mold itself, poor compost, or the combination of the two conditions.

Indicator Molds

Ink Cap Fungi

Common Names:
ink caps, ink weed, wild mushrooms

Scientific Names:
Coprinus fimetarius, Coprinus radiatus, Coprinus sp.

Imperfect Stage:
Ozonium, Rhacophyllus

Coprinus, or ink cap fungi, may appear during spawn run or crop production. Ammonia seems to be a growth requirement of this fungus, and improper management of Phase I and II composting, resulting in ammonia-type compounds, is most often linked with the appearance of ink cap fungi. It has been suggested that variations in the frequency of appearance from year to year may reflect the abundance of ink caps in the straw, cobs, or hay used in compost production, though this has never been proven. Ink cap populations in such crops are probably influenced by composting and growing conditions.

Vegetatively, an ink cap fungus produces a luxurious growth of white fine mycelium in or on the compost before or after spawning. Round white pin initials the sizes of peppercorns ($\frac{1}{16}$-inch diameter) begin to develop on the compost sometimes as early as 3 to 4 days after spawning. Pins develop into mushrooms with narrow white stems and scaly white to gray cone-shaped caps. Once the mushroom forms (Figure 29), it disintegrates quickly into ink black liquid, giving this fungi its name, ink caps. The black liquid characteristic of this genus is the product of autodigestion. Certain ink cap species develop a long fibrous rhizomorph (rootlike structure) that extends into the compost.

Figure 29. After ink cap mushrooms mature, they disintegrate quickly into the ink-black liquid that gives the ink cap fungus its name.

Like *Agaricus*, the fruiting process of *Coprinus* is cyclical, and ink cap mushrooms occasionally may reappear in flushes. More often though, ink cap mushrooms appear only once during the growing process. Once ammonia compounds in the compost are gone, the compost pH decreases, and there is a gradual disappearance of ink caps. Mushroom spawn then will gradually colonize the previously infested compost.

Several species of *Coprinus* occur with the mushroom crop. The larger ink cap, *Coprinus fimetarius*, is characterized by a thick hollow stem and a grayish scaly cap. This mushroom often is associated with severe substrate preparation problems, either during Phase I or Phase II. The smaller species, *Coprinus radiatus,* has a shorter thinner stem and a very fragile pale brown to yellow brown cap. This mushroom often is associated with a breakdown in supplements added at spawning time or a minor composting problem that resulted in ammonia-type compounds being released by the supplement. Other *Coprinus* species have been isolated from mushroom compost, and unnamed species have been reported.

Ink caps may begin to grow as early as the end of Phase II, but more often they first appear during spawn run, after casing, or just before first break. Epidemic infestation of *Coprinus* often is associated with a difficult or poorly managed Phase II composting. Too much breakdown of raw materials during Phase I composting, which affects resiliency or conditioning of the compost, or the addition of too much water, may contribute to a difficult Phase II and residual ammonia compounds. The addition of excessive amounts of inorganic nitrogen to substrate causes an imbalance, which also can result in residual ammonia at spawning time. The thermophilic microflora that grow during Phase II are unable to convert all the ammonia into microbial protein, and the microbes will use up the available carbohydrate or water before the ammonia has been completely converted. These ammonia-type compounds left in the substrate provide food for ink cap development. Spotty or confined occurrences of ink caps in parts of the room suggest that these areas contain compost that is packed nonuniformly or too tightly during the filling operation. High populations of nematodes have been observed in these areas of ink caps, further suggesting that a compacted, tight, or wet substrate was unable to properly heat during pasteurization and the remaining part of Phase II.

Compost moisture may favor the development of ink caps. Overly wet compost is more difficult to condition, partially because of the reduced aeration within the substrate. Excessive use of steam, or steam used to maintain air temperatures during Phase II, when too much fresh air is brought into the room, will cause condensation on the surface of the compost. Excessive condensation will interfere with air and gas exchange from the compost into the air during Phase II. Conversely, dry compost at filling, or excessively high temperatures or ventilation throughout Phase II, will result in moisture becoming the limiting factor for microbial growth. Therefore, the microbes will die before they are able to completely condition or convert ammonia into microbial protein. The resulting ammonia-type compounds provide a food source for growing ink caps.

Ink caps also may grow as the result of improper temperature management during Phase II. Areas of the compost in which the compost temperature did not remain within the range of 115 to 140°F (46 to 60°C) from 72 to 96 hours before and after pasteurization may contain residual ammonia. Oppositely, composts that reheat (recycle) as little as 3 to 5°F (-16 to -15°C) near the end of Phase II will have additional ammonia produced via microbial ammonification of nitrogen compounds. Rejuvenated microbes will use previously formed protein compounds to obtain carbohydrates for their energy, and the nitrogen left from the used proteins may be ammonified. A low air temperature, cooler than 100°F (38°C) and maintained to manage the internal compost temperature, can result in an ammonia-laden layer (0.5 to 1 inch in depth) at the compost surface. In such instances, ink caps can flourish on the ammonia remaining in this surface layer.

Locating the origin of ink caps can aid in deciding why the compost supports ink cap growth. A few scattered ink caps are little cause for concern and may indicate compost nitrogen content at filling time near the limit for a farm. However, a bountiful flush of ink caps suggests excessive ammonia at spawnings and is evidence that certain aspects of Phase I or Phase II composting need to be corrected.

Plaster Molds and Flour Molds

Common Names:
white plaster mold, brown plaster mold, and flour mold

Scientific Names:
Scopulariopsis fimicola, Botryotrichum piluliferum, Papulaspora byssina, Thielavia thermophila, Sporotrichum sp.*, Trichothecium roseum*

Outdated Names:
Monilia fimicola, Oospoio sp.*, Myriococcum praecox*

Perfect Stages:
Dichotomyces (S. fimicola), Chaetomium (B. piliiliforum), Corticium (Sporotrichum), and *Hypomyces (T. roseum)*

Imperfect Stages:
Acremonium, Chrysosporium, Myceliopthora, Sepedonium, Sporotrichum, Thielavia

Although the fungus that causes flour mold is not the same as that causing plaster mold, it is generally believed that the same nutritional factors favor the growth of the two mold groups; so they will be discussed together. Several fungi have been associated with the white and brown plaster mold condition. Briefly, *Scopulariopsis fimicola* probably is the most familiar, and *Botryotrichum piluliferum* is the most recently recognized. Species of *Sporotrichum, Thielavia thermophila,* and *Trichothecium roseum* have been called plaster or flour molds. Brown plaster mold has been used to describe infestations of *Papulaspora byssina, Scopulariopsis fimicola,* and *P. byssina.* The reader is referred to other references to obtain more details on the taxonomy of these fungi.

White plaster mold first appears, near the end of Phase II or during spawn run, as a small irregular patch of white spawnlike aerial growth on the compost surface (Figure 30). Within a few days, this aerial hyphae begins to resemble plaster of paris. Eventually, the aerial growth completely disappears, leaves a white mold on the compost surface, and looks like spilled plaster or flour. In some cases, the white plaster mold grows from the infested area of the compost and looks to be flecks of plaster or flour on the casing surface. Some colonies have a pearly glisten, and the mycelium is creamy white to buff colored instead of snow white. Other plaster or flour molds, species of *Sporotrichum* and *Trichothecium roseum,* appear initially as fluffy white molds that develop a light peach color and light rose-pink color, respectively.

Figure 30. White plaster mold first appears as a small irregular patch of white spawnlike aerial growth on the compost surface.

Thielavia thermophilia is thermophilic (heat loving), and for this reason is unique among indicator molds. *Thielavia* grows rapidly and abundantly during the last days of Phase II, and is first observed as circular- to oval-shaped patches of fluffy white mold, 1 or 2 feet in diameter, on the compost surface. Before spawning, spores in the colony center start to mature, and the fluffy texture of the mold takes on a granular, flourlike appearance. Color changes from white to salmon pink and then to beige. A few days after spawning, the white fluffy growth of this mold again may appear salmon pink to beige-colored. The colonies may grow densely and rapidly through the compost, eventually colonizing in large areas or in many areas within the room. The powdery masses of spores become airborne when the infested area is

disturbed. Near the end of the crop cycle, areas infested by this mold usually contain numerous small black spherical fruiting structures in addition to the fluffy beige form. These fruiting bodies are the sexual stage of *T. thermophila.* Growth of this mold in compost occurs in conditions similar to those that favor the growth of other brown molds and ink caps. It is possible that if compost conditions are conducive to the growth of one of these molds, several types or species may be growing in close proximity to each other.

The rapid growth of *T. themophila* in infested areas may increase compost temperatures to a range as high as 105 to 120°F (41 to 49°C) and prohibit or kill spawn growth. Once the mold has used up its food, the compost cools, and spawn often recolonizes the infested areas if ammonification of the compost has not occurred. However, these areas often fail to support either a vigorous spawn growth or high yields. This white mold develops in restricted spots and has not been observed infesting an entire tray or bed of compost. Consequently, high compost temperatures are encountered only in these spots, and routine monitoring of compost temperatures during spawn run may not reveal the presence of "hot spots" caused by *T. thermophila.* Presence of this plaster mold is noticed most often during a visual inspection of spawn growth development. It may be detected on farms where compost temperature is monitored in a great number of locations daily. Most other plaster and flour molds that occur in mushroom compost do not cause "hot spots."

The brown plaster mold fungus, *Papulaspora byssina,* first appears on the compost surface during the spawn run. Dense plasterlike white mold may develop in areas 6 to 15 inches in diameter. As the fungus matures, the center of the colony changes from white to yellow or tan, and then to brown, orange, or rust color. Brown plaster mold colonies grow a bit above the compost and often are outlined by an actively growing outer fringe of white mycelium. Colonies tend not to be fluffy in structure. Several colonies can grow together to form a continuous coating over the surface of the compost or on damp bedboards. After casing, the mold may grow up through the casing and emerge on the surface. The mold usually is white at first, and the color may change to the typical brown with a white fringe. These molds are easily recognized by hand lens as a mass of darkly pigmented spherical structures on the compost straws or casing. The beadlike structures, called "bulbils," appear and are interwoven with a fine network of white hyphae.

It is currently thought that growth of plaster molds and flour molds occurs where compost is too broken down or overly wet during Phase I composting and/or inadequately or improperly managed during the Phase II process. These molds develop in mushroom compost when nitrogen sources, formed during Phase I, are left after Phase II. These nitrogen-type compounds are not converted into microbial protein, are referred to as amines and amides, and most often appear in composts with pH levels above 8.5.

Long composting time, which results in overly composted manure, is more apt to support the growth of these plaster and flour molds. Plaster or flour molds will appear in a facility when improperly conditioned compost is made. Although the spawn will grow, conditions that support widespread growth of plaster or flour molds will not support maximum yields of mushrooms. Modification of composting practices to improve compost quality usually reduces the occurrence of flour and plaster molds.

Olive Green Mold

Spores of the olive green mold fungus are heat tolerant and may survive at 140°F (60°C) for 6 hours. However, this mold appears in compost where Phase II ventilation is inadequate. Improperly managed Phase II aeration that leads to an inadequate oxygen level and compost temperatures greater than 142°F (61°C) seems to promote the formation of compounds that appear toxic to spawn growth but favor growth of olive green mold.

An inconspicuous grayish-white fine mycelium growing in compost, or a fine fluffy aerial growth on the compost surface several days after spawning are the early signs of this fungus (Figure 31). Spawn growth is often slowed and reduced during the early part of the spawn growing period. Later in spawn run, this mold's fruiting structures may look like very small gray-green cockleburs or peppercorns about $\frac{1}{16}$ inch in diameter. Fruiting structures are most likely to develop on straws in isolated spots in the affected compost. Compost may have a musty odor and often does not support mushroom spawn growth; therefore, it is common to see olive green mold in black compost that is not colonized by mushroom spawn. The fluffy white-grayish growth or green furry burs characteristic of olive green mold are obvious even on compost colonized by mushroom spawn.

Characteristically, burs are olive green in infested compost, in contrast to the blue-green spore masses of *Penicillium* mold, or the forest-green *Trichoderma* molds.

Once it has been formed in the compost, olive green mold persists throughout a crop. Spawn usually grows into areas occupied by *Chaetomium,* although spawn growth often is delayed. Compost conditions conducive to a widespread infestation of olive green mold may reduce spawn growth significantly, with a coincident reduction in mushroom yields.

Compost that has a good structure, such as that which is resilient when compressed or not overly decomposed during Phase I, will allow for better aeration during Phase II. Adequate air exchange throughout the entire Phase II is necessary to prevent compost from becoming anaerobic. Even a few hours of too little air sometimes is enough to cause compost to become anaerobic and conducive to olive green mold growth. The proportion of outside air introduced into a room to ensure aerobic conditions in the compost throughout Phase II varies from facility to facility.

Excessive compaction or oversaturation of compost with water at filling time should be avoided. Proper manipulation of steam valves, fresh air dampers, doors, and high-speed exhaust or intake fans can ensure the availability of enough air to the compost during Phase II. These procedures also enhance aerobic thermogenesis in the compost, which enables compost temperatures to remain hotter than the air temperature during Phase II. Air temperature and air volume should be managed to maintain a temperature differential and gas exchange between the compost and the air.

Figure 31. Early signs of olive green mold are an inconspicuous grayish-white fine mycelium growing in compost, or a fine fluffy aerial growth on the compost surface several days after spawning.

Black Whisker Mold

Common Names:
black or gray whisker mold, whisker mold

Scientific Names:
Doratomyces microsporus,
Doratomyces stemonitis,
Doratomyces purpureofuscus,
Trichusus spiralus

Outdated Names:
Stysanus stemonitis

Perfect Stages:
Periconia and *Cephalotrichum*

Black whisker mold may occur in compost during spawn run or after casing. It first appears in spawned compost as an erect, black, whiskerlike structure. The distinctive black whisker appearance develops as the spores are maturing (Figure 32).

Black whisker mold fungus in compost indicates an unbalanced nutritional base in the compost at spawning time. When *Chaetomium* green mold is present, black whisker mold also will be present, since both are celluolytic, or fungi that feed on cellulose.

Black whisker mold grows rapidly through the compost at the end of Phase II and at the beginning of the spawn run. Heavily infested areas of compost appear darker than usual because of the masses of black powdery spores. When disturbed, these spores are liberated and the compost appears to be "smoking."

Black whisker mold is not thought to be a serious competitor of mushroom spawn. Its presence usually indicates that the straw has been incompletely decomposed or caramelized. Low Phase

Figure 32. Black whisker mold first appears in spawned compost as erect black whiskerlike structures, highly magnified in this photo. The descriptive black whisker appearance develops as the spores mature.

I temperatures result in excess carbohydrates that are easily used by black whisker mold. Black whisker mold also may indicate that nitrogen supplementation of fresh compost ingredients was inadequate, or conversely, that the proportion of carbohydrates was too high. It has been reported that this mold also grows in compost that overheated during spawn run.

Whether spores of black whisker mold survive peak heat is not known. Growth of *Aspergillus* and *Penicillium* molds also are favored by conditions conducive to the growth of black whisker mold, and these fungi also may be present in the compost. Black whisker mold, *Aspergillus,* and *Penicillium* are mold fungi, which produce abundant numbers of spores. Air heavily laden with spores from these fungi, often erroneously called "gas," can induce an acute allergy-type response in dumping crew personnel. Workers may report respiratory troubles often characterized by asthmalike symptoms including nasal or throat irritation, chest congestion, breathing difficulty, nosebleed, or alternating fever and chills. The response is transitory, but a person sensitive to these spores becomes more sensitive with each exposure, and the discomfort may become more intense. Sensitive or sensitized workers should be assigned tasks elsewhere, away from compost dumping. Proper preparation of compost precludes the development of these molds, so these molds are unknown at many facilities.

Smoky Mold

Common Name:
smoky mold

Scientific Name:
Aspergillus spp.; *Penicillium* spp.*;
Penicillium chermesinum*

Several species of *Penicillium* have been reported in mushroom compost, and most are harmless to the spawn and overall yield; yet, it recently has been reported that *P. chermesinum* has caused serious crop losses when introduced into Phase II compost at spawning time. Symptoms begin to show up as edge breaks at first break. Digging into infested areas causes large clouds of spores to form; hence the name "smoky mold." *Aspergillus* and *Penicillium* often are greenish in color, whereas *P. chermesinum* is characteristically white at first, then turns brown. All smell moldy.

Reported incidence of *P. chermesinum* occurs mostly in bulk Phase I and II systems. Other smoky molds can be found in all systems. It has been suggested that *P. chermesinum* has originated from dirty straw and from other *Penicillium* spp. *and Aspergillus* in overheated, supplemented compost after spawning. A large *P. chermesinum* spore load infecting compost at spawning has the most devastating effect on yield. Much like *Trichoderma* green mold, there may be an interaction between the mycelium of this mold and *Agaricus.* It has been suggested that spawn is either parasitized or effectively repressed in smoky mold. Control of this particular mold is similar to virus control; therefore, extreme hygiene and spore exclusion is essential. However, the spores are quite small, so HEPA filters are required to remove these two-micron spores. Cleaning before and after spawning is essential.

Other smoky molds often are found in compost where less protected spawning supplements are present and overheat during spawn run or after casing. Even brief periods of temperatures above 90°F (32°C) can damage or kill the spawn. These *Penicillium* and *Aspergillus* molds easily colonize the dead spawn grains and supplements. Compost in these areas is generally black at casing or sometimes has a mosaic appearance. Often, these black areas appear toward the center of the beds, where temperatures are warmer (Figure 33). If the overheating occurs within 2–3 days after spawning, residual bacteria may cause compost to begin heating. Often, compost may smell clear of ammonia at spawning, but it will not be completely conditioned. These residual compounds provide food to the bacteria or other mesophilic (heat-loving) microbes. Control of these molds is ensured by compost, which is maintained in the conditioning range during Phase II until it is completely conditioned. It is also important that enough, but not too much, moisture is in the compost. Dry compost may result in the microbes running out of water before they have completely used all the available nitrogen. Conversely, wet compost prevents proper aeration within the compost and prevents the microbes from growing.

Figure 33. Compost in smoky mold-infected areas is generally black at casing, or sometimes has a mosaic appearance. These black areas often appear toward the center of the beds, where temperatures are warmer.

Oedocephalum Mold

Brown mold may appear occasionally as early as during cooldown, before spawning, but more often develops during the latter part of spawn run. The mold first forms irregularly as a light gray mold growing on the compost surface; but within a few days, spores form and begin to mature, and the color changes to dark tan, fawn, or light brown. The growth habit of *Oedocephalum* brown mold varies from a weak growth over the compost surface to a dense coating on the compost straws. This mold grows on compost most of the time, but occasionally it is seen after casing. After casing, *Oedocephalum* grows slowly from sites of infestation up through the casing and may appear on the casing surface before pin formation. The pearly-white mycelium of *Oedocephalum* grows loosely over the surface, but its color changes to silvery brown as the fungus ages and the spores mature (Figure 34).

The appearance of this fungus, discernible through a hand lens, consists of an erect spore-bearing structure with a globular cluster of large spores at its top end. Rubbing *Oedocephalum* brown mold between the thumb and index finger produces a gritty sensation similar to that experienced in rubbing fine sand. This gritty characteristic distinguishes *Oedocephalum* sp. from other white-brown molds in mushroom compost or on casing. Spores of *Oedocephalum* sp. are common in most mushroom composts, but they lie dormant unless induced to germinate

Figure 34. The pearly white *Oedocephalum* mycelium, discernible through a hand lens, consists of an erect spore-bearing structure with a globular cluster of large spores at its top end. Its color changes to silvery brown as the fungus ages and the spores mature.

and grow. The environmental and nutritional conditions that encourage growth are not fully understood. Usually, *Oedocephalum* brown mold growing in compost indicates that ammonia and amines were not completely eliminated during Phase II and are serving as a food for this organism. Growth of *Oedocephalum* does not inhibit spawn growth, but conditions favoring its growth are not optimal for mushroom production. Compost conditions similar to those described for plaster molds are associated with the growth of *Oedocephalum* brown mold.

C. Pest Species Biology and Control

4. Bacterial Diseases

Paul Wuest

Bacterial Blotch—*Pseudomonas tolaasii*

Description

Pseudomonas tolaasii, the cause of bacterial blotch, is an aerobic, non-spore-forming fluorescent bacterium in the genus *Pseudomonadaceae*. It is a common bacterium; many fluorescent Pseudomonads are readily isolated from field soil. These groups of bacteria are rather closely related and often difficult to distinguish, although a unique feature of the species *P. tolaasii* is its ability to infect and discolor commercial button mushrooms. The discoloration is pale yellow at the start and darkens to a golden yellow or rich brown color. The blemishes are superficial but decrease the eye-appeal of mushrooms and lower their quality in the marketplace. This bacterium is not a threat to human health.

Control

Managing bacterial blotch disease on mushrooms is a matter of chlorinating the irrigation water applied to the crop to a concentration of 150 ppm chlorine; using water that is potable (drinkable) as a source for irrigation water; and most importantly, inducing the caps of the mushrooms to dry after an application of irrigation water. It is common to include a 2- to 3-hour drying cycle in environmental management after irrigation. During this time, the ambient temperature should be raised a few degrees, the humidity should be lowered to below 85 percent, and the total airflow should remain unchanged or increased by 10–15 percent. The goal is to lower the humidity in the growing room to induce the mushrooms to dry.

Experience suggests that when the mushroom compost is too dry when it is spawned—less than 60 percent H_2O—the above steps will not eliminate bacterial blotch from the crop. Also, when the source of the peat moss used to case the mushroom beds has changed, bacterial blotch may not be controlled, because some peats foster *P. tolaasii* more than other peats. Another environmental situation in which bacterial blotch is almost impossible to control is when the external air temperatures are moderate (59 to 72°F, or 15 to 22°C) both day and night, and the air is full of water vapor. In such a situation, the condenser of the air conditioner does not turn on, since the air temperature in a growing room is what the grower specified. Since the mushroom growing temperature requirement has been satisfied, the moisture in the outside air is not condensed on the cooling coils. In such instances, placing an electric light close to the air temperature sensor will cause the control system to register that the incoming air is too warm. The condenser will begin to operate, which will remove some of the excessive water from the incoming, ambient air.

Strain Choice

There are a few reports that some wild species of *Agaricus bisporus* possess resistance to bacterial blotch. In addition, there are differing levels of susceptibility among the commercial strains of hybrid white and hybrid off-white mushrooms. Growers may be wise to try different strains to determine response to bacterial blotch, selecting the strain that performs best in the overall conditions at the facility. However, choosing a strain of *A. bisporus* based exclusively on its susceptibility to bacterial blotch may not be in the best interests of production at a facility. Managing bacterial blotch is not simple, and sometimes the best efforts fail. This approach allows a producer to choose a strain well suited for the unique environmental conditions at each facility.

Mummy and False Mummy—*Pseudomonas species*

Description

Mummy disease is characterized by mushrooms that develop to the button stage or larger, then stop growing. The affected mushrooms sometimes develop a curved stipe with translucent, longitudinal streaks on the inside. The mushroom tissue becomes mummylike in appearance: spongy, dry, and leathery. With an early onset of mummy disease, first-break mushrooms will be delayed in their development by a few days, but a break of mushrooms does develop and can be harvested. Second-break mushrooms in the same location exhibit the full-blown symptoms of mummy disease. Thereafter, mushrooms no longer will grow in that area. The poor quality of the mushrooms and the lack of subsequent harvest from infected areas can create a severe economic loss.

The causative agent that induces mummy disease is a bacterium, a species of *Pseudomonas* closely related to but not the same as the bacterium that causes bacterial blotch. Many Pseudomonas bacteria commonly are found in and on organic matter, so it is doubtful the mummy bacterium is a unique organism introduced from outside a mushroom farm. Rather, the mummy bacterium may be a normal part of the bacterial microflora of most mushroom composts. When conditions favor its growth and reproduction, its population grows large enough to cause the disease recognized as mummy.

A scientist working at a cave farm in Missouri in the 1930s first described mummy disease. It appeared as a reasonably large patch of mummified mushrooms on first break, with the size of the affected area getting larger as the crop aged from break to break. This symptom pattern continued until the middle 1970s, when off-white strains predominated at mushroom farms, and into the 1980s, when hybrid white and hybrid off-white mushroom strains were the most widely grown strains of *A. bisporus* at mushroom farms. Since then, mummy disease seems to initially affect a few squares (8 to 12 lineal feet) in a growing room at traditional bed farms and does not spread along a bed after it first appears. This newer expression of mummy disease, sometimes referred to as *false mummy*, shows additional symptoms. These include a fuzzy mycelial growth at the base of mushrooms (Figure 35) and very coarse strands (rhizomorphs) attached to the mushrooms when picked. Also, a layer of tissue at the base of the stipe turns mahogany brown or yellow-brown when the stipe is cut longitudinally and exposed to the air for a few minutes. The bed area affected by these newer symptoms increases very little in size from break to break. If the symptomatic area is allowed to dry between breaks, some mushrooms will grow and can be harvested from the affected areas.

An unusual phenomenon has been seen repeatedly when mummy disease appears in a growing room. It is reasonably common for the total production from the room with mummy disease to be equal to or greater than the production from a room where no mummy disease occurs. This oft-repeated observation suggests the bacterium associated with mummy disease may be ecologically related to one or more other organisms that are capable of enhancing production. Or, the conditions that favor mummy disease development also favor the optimum production of mushrooms.

Figure 35. Mummy disease, showing the tilted cap and fuzzy bottom stems.

Control

The effectiveness of mummy control measures may be difficult to predict, though moisture management is the basis for many control efforts. Experience suggests that during times of the year when evaporation from spawned compost or cased mushroom compost is less than it should be, mummy disease develops. Bed growers in Chester County, Pennsylvania, have had experiences in which the addition of water to compost before or at the time of spawning predisposed the compost to supporting mummy disease. Oddly though, bed growers in Berks County, Pennsylvania, added water to compost at spawning without this response. Other factors may be involved: Berks County growers generally used more hay in blended composts, while Chester County growers use more horse manure or straw in their blended composts.

At farms when spawned compost is covered with plastic for the spawn run period, some growers have seen less mummy disease when they cut open and turn back the plastic whenever water accumulates on its underside. This practice prevents the accumulated water from dripping into the compost and soaking the top of the bed.

In the 1970s, when off-white strains predominated, it was common practice to remove the plastic a few days before casing to ensure the surface of the compost was completely dry before casing was applied. The off-white strains were much more sensitive than earlier strains and could be harmed by too much water in/on the surface compost, unrelated to the mummy threat. Mummy misdiagnoses often occurred when compost beds were cased when they were too wet. Under these conditions, spawn growth into the casing was slow, mushroom formation was delayed, and the mushrooms appeared to have the characteristics of mummy disease. In fact, the problem was water stress, not mummy disease.

Another attempt at mummy control was to water the surface of the compost with chlorinated water (150 ppm Cl) a few days before casing. Some growers were confident this practice controlled mummy; an equal or higher number assumed it enhanced the amount of mummy in a crop.

Sanitation and hygiene cannot be overlooked in efforts to control mummy disease. In their absence, mummy-infested compost moving through a tray line can contaminate the equipment, which in turn contaminates the compost moving along behind it. One mummy-infested tray of compost can serve as an inoculum source and infest most of the other compost in one growing room of trays. At tray farms, especially, mummy disease can cause devastating crop losses. Thorough washing and sanitizing of tray-handling equipment is essential to minimize the threat of spreading the cause of mummy disease; the same is true for spawning equipment. Special attention to sanitation and good hygiene in and around spawn bags, spawn, and the spawning process is essential.

Sanitation and hygiene, practiced within an environment where moisture management promotes evaporation from compost and casing, are the only ways to reduce the threat of a mummy disease infestation.

C. Pest Species Biology and Control

5. Nematodes

Phillip S. Coles

Introduction

Nematodes thrive in raw compost and can exist in excessive numbers during the mushroom growing process. While some growers believe that nematodes are merely an indicator that compost and casing preparation has gone awry, it is wiser for growers to assume that nematodes can represent the risk of yield losses, and to take precautions against their proliferation.

Nematodes

Nematodes are tiny, very primitive roundworms. They appeared early on the evolutionary stage, being the first animals to evolve a body cavity. They are extremely abundant in both types and numbers. There are about 12,000 species currently known, but scientific opinion holds that the number of species actually could be 100 times greater. Typically, nematodes range in size from 0.2 mm to 6 mm in length, though some may be much longer.

Nematodes are found in marine, freshwater, and soil habitats. It has been estimated that there are 8 billion nematodes in an average acre of field soil. One square meter of garden soil probably contains approximately 2 to 4 million nematodes. Many are parasites; in fact, almost all types of creatures studied by scientists have at least one species of nematode that parasitizes them. Roughly 50 species parasitize humans.

Caenorhabditis elegans, one of the saprophytic nematodes to be discussed below, has become an important tool for genetic and developmental researchers. This organism is made up of only 1,000 cells. It matures in 3 days and has a transparent body that allows scientists to watch the dividing cells.

Nematodes in Mushroom Growing

It is fortunate that nematodes do no harm in raw compost, because they are ubiquitous in the materials used to prepare the compost mix, and their complete removal, if possible, would be extraordinarily expensive. The richness of the compost environment in terms of food, water, and oxygen provides nematodes with an excellent habitat, at least until composting temperatures reach lethal ranges. The cooler outer portions of the rick, if not mixed and turned into the interior, will continue to support nematode populations into Phase II.

There are four general types of nematodes: parasitic, saprophytic, predatory, and animal parasitic. Only the first two are discussed here. For mushroom growers, the primary difference between these two groups lies in their feeding habits. The parasitic nematode feeds directly on mushroom mycelium, whereas the saprophytic nematode feeds on bacteria, protozoa, fungal spores, and other bits of organic matter, but does not attack the mycelium.

Parasitic Nematodes

These nematodes, also referred to as fungal-feeding or mycophytic nematodes, are increasingly rare in mushroom farming today. Presently, industry choices of casing materials or pasteurization of casing usually avoid outbreaks. In the past, however, they were responsible for disastrous crop losses.

The parasitic nematodes use their stylet (a needlelike mouthpart) to pierce the mycelial cell and inject digestive juices. The same stylet then becomes straw through which the nematode consumes the liquefied cell contents. As nematodes move through the mycelium-filled compost, they first destroy the fine hyphal structures and leave the mycelium looking stringy. Thereafter, larger mycelium is destroyed, leaving small barren bed areas that grow progressively larger as the nematodes venture outward into healthy compost. If the conditions are optimal for the nematodes—moderate temperature (68–77°F, 20–25°C) and wetness—entire beds can be denuded of their mycelium. Depending on the number of nematodes on the bed, the mushroom crop will be reduced or eliminated.

Under good conditions, nematodes can multiply 30- to 100-fold in 2 weeks. When their burgeoning population exhausts the compost of its nutrients, the nematodes respond to the changing environment by swarming to the surface. Exposed there, they can be picked up easily by vectors such as humans and flies. If dried slowly, the nematodes become dormant and can be distributed by even slight air movements.

Compost infested with nematodes has a characteristic appearance: soggy, sour smelling, and depressed. The nematode-trapping gray mold, *Arthrobotrys superba,* may appear in areas where the mycelium has been destroyed. This soggy mess is apparently good habitat for the saprophytic nematodes, the second of the two types discussed here, for they frequently appear in these areas.

Saprophytic Nematodes

These nematodes, often referred to as "free-living," now are more commonly associated with mushroom farming than the parasitic species. They characterize poorly prepared compost and/or casing and cause severe deterioration of mycelium in their own right. The common saprophytic species are listed below. In most cases of infestation, two species of these nematodes are present.

Common Saprophytic Nematode Species

Acrobeloides apliticus

Acrobeloides buetschii

Caenorhabditis elegans

Cruzenema lambdiensis

Panagrolalmus rigidus

Pelodera (Pelodera) strongyloides

Rhabditis (Cephaloboides) oxycera

Rhabditis (Choriorhabditis) longicaudatus

Rhabditis (Rhabditis) terricola

Rhabditis (Pellioditis) pellio

Saprophytic nematodes' feeding habits differ markedly from their parasitic counterparts. They suck in and chew the particles of food they consume. They possess a muscular pharyngeal bulb, which creates the suction to draw in food particles and liquids. The saprophytic nematodes multiply even faster—one hundred-fold in 3 days—than those that are parasitic. Many of these nematodes are parthenogenetic (self-fertile).

At low levels, these nematodes have little effect on mycelium. As the nematode numbers increase, mycelium begins to grow slowly and weakly. At high infestation levels, the strands completely degenerate. Research suggests that the extent of the saprophytic nematode damage to mycelium is closely tied to the number of bacteria, the nematodes' primary food source, present in the spawned compost or casing. Their detrimental effects on the mycelium appear to be linked to the release of a toxin or byproduct into the compost. Extracts taken from diseased compost and casing show there is greater crop damage when both bacteria and nematodes are present in high numbers than when only bacteria are present. The enhanced crop injury may be the result of increased production of toxins when both are present, or may reflect some way in which the nematodes make possible a more rapid or thorough bacterial colonization of the compost.

There is an interesting ecological relationship among the nematodes, bacteria, and mycelium. Under excessively wet compost conditions, bacteria have an advantage over mycelium, and as the nematode food source, their increase in numbers encourages the expansion of the nematode population. The high numbers of bacteria also inhibit normal growth of mycelium. The compost deteriorates and becomes wet and increasingly anaerobic. Under less wet conditions, the mycelium can spread, use the water for its own growth, and dry out the compost to a point that inhibits the bacterial proliferation. The nematodes remain in low number because of the dry conditions and the limited food source. The environment remains favorable for mushroom production.

Effects on mushrooms can range from little damage to total elimination of the crop. The appearance of the compost can gives clues to the damage to come; when dark, watery, barren patches develop, production will be severely affected.

Survival Characteristics

Nematodes owe their abundance and widespread distribution in part to their remarkable survival abilities. If dried slowly, they enter a heat-resistant dormant state that can persist for years until they contact enough moisture to break dormancy. In the dried state, they are distributed easily by air currents. They also can survive without food for months. They are not susceptible to cold or freezing, and they regain their vigor once temperatures are more moderate. When their high numbers begin to deplete the readily available food supplies in compost, they show a collective swarming behavior that brings them to the compost surface for a greater chance of dispersal. The saprophytic nematodes take this group behavior a step further and form into columns of living nematodes, hundreds strong, that wave about on the surface of the mushroom bed, ready to adhere to hands, tools, flies, or other objects. This phenomenon is called winking. The waving strands of winking nematodes can be observed by holding a flashlight at a 45-degree angle to the bed. A grower's IPM plan should take into account these survival traits to minimize the opportunities for nematode dispersal.

Sources of Inoculum

Nematodes are carried into the growing process in a number of ways; the most obvious of which is in the compost. As noted above, nematodes are associated with raw materials entering the compost yard and respond by proliferating in the initially favorable compost environment. On the cooler parts of the rick, inside clumps of compost materials, or in excessively wet compost, they may survive Phase I and enter Phase II. The vast majority of their population may be destroyed in a Phase II room during pasteurization at about 140°F (60°C), but survivors can persist in wet areas, dry areas, and clumps, and can continue on to spawning. There they encounter a more favorable environment: temperatures around 75°F (24°C), moist conditions, and near-neutral pH (7.5). Mixing at spawning distributes the nematodes. The spawning machine can contaminate many subsequent beds or trays after spawning a single infested batch of Phase II compost.

Peat, as it is introduced into the growing process, is dry, has a low pH, and usually does not contain nematodes. Once in place as casing, however, as conditions become more moist, peat provides a favorable environment. Compared to compost, casing provides a habitat with less interference by mycelium. Pasteurization temperatures, as noted above, and careful watering (moist but not wet) can reduce or eliminate nematode populations; but failure to manage these environmental conditions can allow nematodes to move further into the growing process.

Another source of nematodes in a crop is a preceding infested crop. In growing rooms, woodwork and ceiling insulation contaminated with nematodes can inoculate successive crops. If high temperatures during pasteurization do not penetrate into the wood, especially into the cracks and crevices, nematodes will not be destroyed. Likewise, moisture dripping from contaminated ceiling insulation spreads nematodes to new beds.

Nematodes can invade growing rooms from other areas of production in a variety of ways. Dust can carry dormant nematodes between rooms, and flies, mites, hands, boots, and tools can carry nematodes acquired from contact with swarms or contaminated materials. Equipment such as spawning machines, if not designed for easy cleaning and if not routinely sanitized, provide nematodes with an effective means of distribution.

Sampling, Separation, and Identification

Ricks, trays, or beds that are suspected of infestation can be tested for presence and relative quantity of nematodes. In any testing procedure, the integrity of the sample is critical; in this case, the location at which the sample is collected can strongly influence the results. Collecting samples from the hottest portions of the materials usually will give negative results because nematodes rarely survive there. Sampling at cool locations or areas where heating has been nonuniform in the past is more likely to produce detection of the pests. Incubation of samples sometimes is necessary to provide adult nematodes for identification.

Nematodes can be separated from compost or casing and identified visually. A Baermann funnel (Figure 36) is a convenient tool for collecting nematodes for further investigation. The apparatus consists of a support system that holds a funnel with a bottom tap closure. The funnel is filled with fresh water. A cloth or strong, fine mesh bag is suspended over the water at the top of the funnel. Strong commercial tissues will work. A sample of compost or casing is placed into the bag. The nematodes move out of the sample, and since they are slightly denser than water, sink down the funnel until they are stopped by the tap closure. The tap closure is opened after the migration has proceeded for several hours, and the nematodes are collected in a shallow glass dish.

Nematode identification by nonexperts is limited to differentiation of general type or genus level. The most useful distinguishing characteristic is the appearance of the anterior (front) end where the mouthparts are located. A

Figure 36. A Baermann funnel is a simple tool for extracting nematodes from material.

blunt end from which the needle-like stylet can extend shows the organism to be parasitic. Saprophytic nematodes lack the stylet and appear to have liplike bulbs stuck on their anterior ends. By noting the structures of the anterior end, the shape and size of the internal structures, the body length and other features, and contrasting them to published illustrations of nematode types (rhabditoid, aphelenchoid, etc.), the nematodes' identity can be deter-mined well enough to make control judgments or to process comparisons. But, for the grower, simply the existence of nematodes, regardless of the species, is a problem.

Control Measures

Because nematodes are ubiquitous, total prevention of nematode invasion and total eradication during infestations is unlikely. Further, since nematicides are not available to mushroom growers, measures to prevent or control infesta-tions are limited to ensuring that normal temperature and sanitation safeguards are followed, and that enhanced measures are instituted when necessary. A well-prepared IPM pro-gram should outline clearly these measures and help keep all members of the growing team on track.

The Phase I rick provides the grower with his first opportunity for nematode control. Good Phase I temperatures, uniform mixing of the raw materials, and ensuring that cool shoulders are moved to the interior positions are measures that will reduce the number of nematodes moving on to Phase II.

Compost should be pasteurized at 140°F (60°C) for 2 hours to subject the nematodes to killing temperatures. This temperature range is adequate to kill wet nematodes, but if the compost and nematodes dry out, temperatures as high as 160°F (71°C) would be required for lethal effect. The compost may require moisture adjustment to avoid being overly dry, but care should be taken not to make the compost soggy, or bacterial development will be encouraged. The heating system should be checked and compost temperatures monitored to verify that uniform peak heats are reached in all areas of the compost. Casing should be pasteurized at 140°F (60°C) unless the grower is confident that each shipment is free of nematodes. Recontamination of the casing should be avoided.

At spawning, any compost suspected of harboring significant nematode populations should be processed last and followed by scrupulous cleanup of machinery. During cropping, the grower can do little to control an infestation other that to prevent further spread. One of a grower's challenges is to rid the growing room of its legacy of nematodes. Pasteurization at temperatures of 160°F (71°C) should be conducted.

Scrupulous attention to sanitation throughout the growing process, the hallmark of a properly maintained farm, will contribute greatly to nematode control. In addition to typical sanitation practices, the following should be considered:

- Develop and enforce rules restricting personnel movement between compost areas and growing rooms.

- If trays, shelf boards, or other such items cannot be pasteurized, they can be washed with a steam pressure washer—though this treatment may be insufficient to kill all nematodes. Sanitizers should be used in rooms and on equipment. This is especially important on floors, since they act as a heat sink during pasteurization and rarely can have their temperatures raised sufficiently to kill nematodes.

- Redouble sanitation efforts at spawning. Review spawning machine design and positioning to determine if better cleaning is possible with adjustments or retrofitting.

- Take precautions against nematode spread by bits of spilled compost from infested trays. Clean up any dropped materials before they are carried into noninfested areas.

These control measures should be used as the basis of a grower's control program, but in certain cases, infestations appear that require remedies tailored to the particular farm operation or even the season of the year. The two case histories below illustrate creative problem solving in nematode control.

Case History #1: Ross and Burden

(as presented in "An unusual problem—saprophagous nematodes." *Mushroom News.* January 1982.)

Ross and Burden describe sudden, massive crop losses that were traced eventually to saprophytic nematode *(Rhabditis)* infestations. In the first occurrence, the farm's production dropped 50% in two weeks, and in a later instance, dropped to 10% of budgeted levels. Ross and Burden observed lost production in the first break, with barren patches appearing on some trays. Other trays were completely barren. Mycelial degeneration was evident, pinning was poor, and large numbers of nematodes became visible.

Their investigations uncovered, among other things, that the production problem was worse on trays that were watered at spawning, and that significant numbers of nematodes were surviving peak heat. After further investigation, they concluded that active compost (which also had a higher-than-normal initial load of nematodes) required extra fresh air in Phase II. However, the air/bed temperature differential was sufficient to dry and cool the surface of the compost, a condition that protected the nematodes from heat kill. Flooding during the same period likely had carried many nematodes into the farm, providing the unusually high pest pressure. The coincidence of the extra burden of pests and the surface drying in Phase II set off the chain of events that resulted in catastrophic infestation. They reported, "A few simple anti-nematode measures were put into operation, and the farm yield recovered very quickly. . . ."

This incident and another nematode problem with a shelf operation provided the investigators' impetus to delve further into the factors influencing nematode infestation. They concluded that saprophytic nematodes could be a primary cause of crop losses.

Case History #2: Barber and Cantarera

(as presented in "Seasonal nematode problems." *Mushroom News.* June 1987.)

Barber and Cantarera described a common phenomenon, occurring in late winter and spring in southeastern Pennsylvania, of dramatic increases in nematode populations in crops ready to pin. They suggested a cause and described measures to remedy the problem.

The phenomenon, they wrote, has its origin in the cold shoulders found in compost ricks during the winter months. If effective cross-mixing of the rick is not accomplished, these regions fail to achieve the temperatures necessary for nematode kill. This extra load of nematodes is carried into Phase II, where routine pasteurization practices may be unable to bring nematode populations down to safe levels. Barber and Cantarera suggest that sampling of Phase II trays in locations likely to be cooler (near sideboards, ends, and surfaces) gives a better indication of nematode survival than sampling the centers of trays. When swarming of nematodes is observed before pinning or by first break, the grower can safely assume that Phase II pasteurization has fallen short of expectations. Dryness may be the cause, and moistening the compost surface at fill or just before pasteurization assists the normal peak heats in producing the necessary kill. However, the wetting must be judicious and soaking avoided. These simple measures have mitigated seasonal nematode problems. They warn farmers to avoid the belief that nematodes are "not really harmful."

The Future

The future may hold new options for nematode control. At the present, we have few ways of dealing with nematodes once they are infesting a bed. But new applications of biological control may change that. Nematode-trapping fungi, for example, hold promise for pest control. These fungi can pierce the outer surface (cuticle) of nematodes, invade their bodies, and lay spores on the inside. In the process, the nematode is killed, but before death comes, its movements can disperse the fungi by carrying spores through the compost or casing. Research has shown that *Arthrobotrys irregularis* kills nematodes, but use of this fungus has not developed a wide following.

A great aid to the grower would be the development of strains resistant to the toxins that are suspected of being produced in nematode infestations. Off-white and white strains were shown years ago to react differently to extracts prepared from nematode-infested compost and casing, giving rise to hopes for commercially effective levels of resistance in the hybrid mushroom strains used today. Unfortunately, such strains are not available yet, but their pursuit is a worthwhile venture for researchers.

Conclusion

This section could not end with a better message than that contained in the last sentence of Case History #2. A complacent opinion that will not serve the grower well is that nematodes, especially saprophytic nematodes, are not really pests, or that they are only indicators of other problems. A valid IPM program must consider the potential for nematode infestation and detail all farm-specific anti-nematode measures to avoid expensive crop loss, especially since pesticides are not an option.

C. Pest Species Biology and Control

6. Virus Disease

C. Peter Romaine

Introduction

Of all the diseases confronting the grower, none has been the subject of more confusion than virus disease. It has been known by a variety of names, shows a wide range of symptoms, and in the early stages, can be overlooked completely. Virus disease can be confused with the effect of poor cultural practice. It is increasingly apparent, though, that the economic impact of virus disease is significant and realized worldwide.

History of Virus Disease

Virus disease, which also is known as La France disease, dieback, X disease, watery stipe, and brown disease, was noted first in 1948 by James Sinden and Edith Hauser on the La France Brothers mushroom farm located in southeastern Pennsylvania. The disease was reported later in England and the Netherlands, and probably occurs worldwide.

We now are confident that La France disease is caused by a virus. During the 1960s, Michael Hollings in England proposed that a virus caused this disease. He prepared extracts from diseased mushrooms and found that they contained several types of viruslike particles when viewed at a high magnification with an electron microscope.

The scientific confirmation of the viral cause of the disease did not come easily or quickly. Only after many years of scientific research was the identity of these viruslike particles determined with some certainty. It is now known that there are at least three types of viruses of interest to mushroom growers. La France isometric virus (LIV) is thought to be the main cause of virus disease. Mushroom bacilliform virus (MBV) is associated with the disease, but may be a benign virus. Vesicle virus (VV) also appears to be a benign virus that is widely distributed in commercial mushrooms.

Figure 37. Symptoms of virus disease. Shown is the characteristic "drumstick" syndrome involving elongated stems and small, misshapen caps.

Disease Symptoms

Part of the confusion surrounding virus disease likely resulted from the range of symptoms by which the disease presents itself. The disease can reveal itself in two severity ranges. In its less severe form, the virus causes only minor yield losses. Mushrooms have a normal appearance, although yield may be slightly depressed, and the crop appears to be suffering the effect of poor cultural practices.

In the more severe form, the disease causes a delay in the emergence of mushrooms. When the mushrooms appear, they have small caps and long stems that growers refer to as the "drumstick syndrome" (Figure 37). The mushrooms may look similar to those grown in an atmosphere with an elevated level of CO_2. The mushrooms are poorly anchored in the casing, and their veils open prematurely and discharge spores. Nematodes and lipstick mold may be abundant in the compost, indicating inadequate and nonuniform peak heats in Phase I and Phase II. In many cases of severe virus disease, the casing shows spots completely barren of mycelium; these areas fail to develop mushrooms. This "dieback" syndrome probably is related to high populations of nematodes rather than to the direct effects of viral infection.

While the severe form of virus disease is dramatic, much of the economic impact is caused by the yield loss associated with the less severe form. This yield depression can occur early on, before the grower suspects virus disease has effected a crop. If the slight yield loss is noticed at all, it may be confused with the effect of suboptimal cultural conditions. It is critical that the grower arranges for clinical testing of the crop for the presence of virus, to confirm the existence of the disease once it is suspected.

Observations over the years have given clues to the factors influencing the severity of the disease:

- Generally, the greater the square footage of a bed showing disease symptoms and the earlier the symptoms appear, the greater the crop loss.

- The closer infection occurs to the time of spawning, the more severe the disease. Contaminated compost produces a severe infection if mixed into noncontaminated compost at spawning.

- The earlier in the cropping cycle infection occurs, the greater the crop loss, since most mushrooms appear in the early breaks.

- All mushroom varieties are susceptible to virus disease. There is anecdotal evidence that brown strains are more resistant than off-white and white hybrid strains, although the purported degree of resistance has never been quantified. In the absence of resistant strains, control of the disease remains in the hands of the grower.

The Viruses

A trained researcher can detect many kinds of viruses "lurking" in mushrooms, but very few viruses have any known impact on the appearance or growth of the mushroom. The same is true in all the organisms that scientists have studied. We also harbor many viruses in our bodies that seem to have no discernible effect on us at all, and we are concerned only about those that cause disease.

The mushroom viruses that have been studied so far are composed of the genetic information-encoding chemical ribonucleic acid (RNA). A protective protein coat covers the RNA, but in the case of the VV, a lipid membrane replaces the protein shell.

Viruses multiply to astronomical numbers within the cells of their host, but they lack the biochemical features to do so on their own. Consequently, they must reproduce inside a host cell; in effect, they take over the operations of the cell for their own multiplication. For this reason, viruses are considered nonliving "molecular pirates." For the same reason, mushroom viruses are not transmitted as "free-living" particles within the compost, casing, or water, but rather are transmitted only from within a living organism (i.e., mushroom spores and mycelium).

LIV is the infectious agent that has been implicated in virus disease. LIV is found in all virus disease-affected mushrooms. MBV is not considered a cause of virus disease. Research has shown that MBV is present in some healthy mushrooms, but it also is present in most, but not all, mushrooms affected by virus disease—but never without LIV being present too. LIV seems quite capable of causing disease without the assistance of MBV. It is suspicious, however, that both MBV and LIV are detected in most cases of virus disease. MBV conceivably could modify the severity of the disease symptoms or may cause another type of disease not yet described. Or, it may have no effect on the mushroom whatsoever. VV occurs in both healthy mushrooms and those showing virus disease symptoms, and for this reason is thought to be benign.

The table below summarizes our current understanding of the occurrence of these three viruses in healthy mushrooms and in mushrooms showing symptoms of virus disease.

Virus Type	Incidence of the Virus in:	
	Healthy Mushrooms	Diseased Mushrooms
LIV	<1%	100%
MBV	~5%	~60%, but only with LIV
VV	100%	100%

Sources of Infection

LIV associated with virus disease is transmitted through spores and mycelium of the mushroom. Transmission of the virus by infected mushroom mycelium in the compost was investigated even before the viral nature of the disease was known. It was shown that infected mycelium could, if introduced experimentally or accidentally on untreated wooden surfaces, carry the virus into a healthy crop. The healthy crop then would develop the symptoms of the disease. Virus transmission by way of infected mycelium has been associated with severe disease outbreaks, possibly owing to the ability of the mycelium to fuse quickly with the healthy spawn and transmit the virus.

Spores now are believed to be the most important mechanism for spread of the virus. Spores are produced in prodigious numbers within mushroom farms. One mushroom can discharge 1.3 billion spores, and spore discharge rates from exhaust air can be as high as 3.7 billion per minute. Though diseased mushrooms produce fewer spores than healthy ones, almost 70 percent of the viable spores discharged by diseased mushrooms contain LIV. When a diseased spore germinates in compost, it sends out its own mycelium, which can infect healthy mycelium (i.e., spawn). As rhizomorphs and mushrooms develop from the infected mycelium, the virus multiplies and spreads throughout the tissues, causing disease and infecting the new mushrooms and their spores. Picking mushrooms tight stops this disease-spreading cycle by preventing the release of spores. Since diseased mushrooms tend to open and release their spores prematurely compared to healthy mushrooms, growers must be very diligent in their picking practices.

Spore-transmitted virus disease tends to be less severe than mycelium-transmitted virus disease, possibly because of the extra time necessary for spores to germinate before infecting mycelium and spreading throughout a crop of mushrooms. This delay is somewhat offset by the large number of spores available to carry the virus.

Patterns of Infection

Now that virus disease is recognized in its various forms, growers have been able to discern certain patterns of infection. Historically, the disease commonly flared up when construction on the farm disturbed a settlement of virus-infected spores. Typically, growers observed the disease in crops that were being spawned at or near the time of the construction. More recently, outbreaks have been associated with a composting problem. Much like an indicator mold, virus disease shows that the compost is not being heated uniformly to temperatures that are high enough to destroy the sources of the virus in the compost.

Clinical Diagnosis of Virus Disease

Clinical diagnosis of virus disease can be a critical part of an IPM program. As noted earlier, clinical diagnosis of viruses in a crop is an important first step in correcting an outbreak, especially when the disease is manifesting its milder form. Testing for virus disease is useful in other ways. The grower should routinely test to determine if virus disease is present but unnoticed in crops. This precaution can pay for itself in reduced yield loss. Once a virus outbreak occurs, the grower can use virus testing to monitor the course of the infection to verify that control measures are successful. How this testing is performed has changed and improved in recent years. Testing is now extremely sensitive, available, and affordable. Some commercial spawn manufacturers offer virus diagnosis as a customer service.

Early on, viruses were detected visually using an electron microscope alone or in combination with antibodies (immunosorbent electron microscopy) that captured the virus particles, making them easier to detect. This testing procedure is expensive, but it is still used in parts of Europe.

Testing for the presence of certain double-stranded RNAs (dsRNAs), the genetic component of the virus, was widely practiced in the 1980s and was the research tool that led to the identification of LIV as the virus disease agent (Figure 38). This test was used at farms to diagnose, detect, and monitor virus outbreaks. Its use allowed growers to track progress through the course of outbreaks, match results with control practices, and verify when virus outbreaks actually had disappeared.

Figure 38. Clinical diagnosis of virus disease. DsRNA analysis (left) and RT-PCR analysis (right) of healthy (Hea) and diseased (Dis) mushrooms. DsRNA analysis detects a vesicle virus dsRNA in healthy mushrooms, and numerous La France isometric virus dsRNAs in diseased mushrooms. RT-PCR detects only La France isometric virus in diseased mushrooms with the presence of a specific DNA product (arrow). DNA size markers are shown (Mkr).
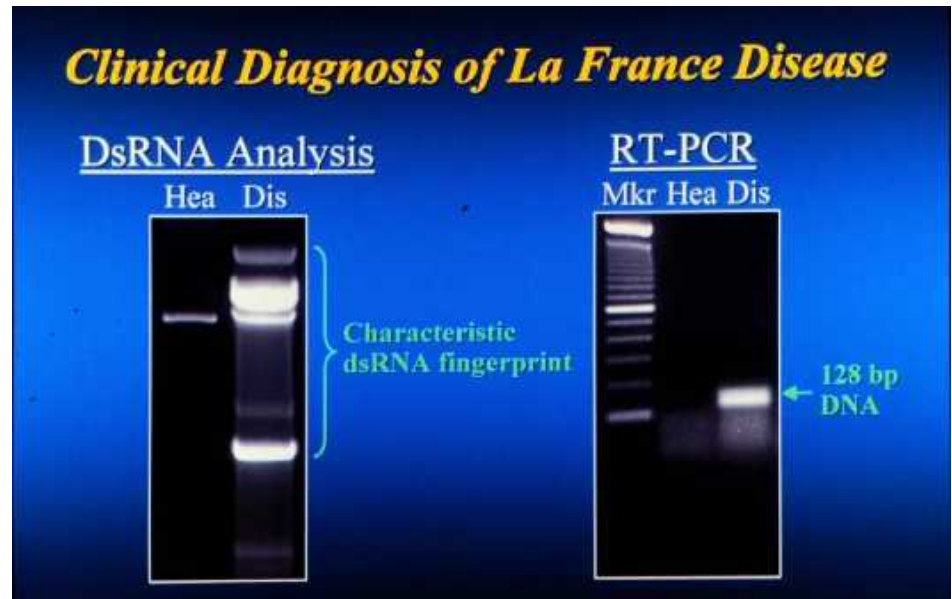
Reverse-transcription polymerase chain reaction (RT-PCR) is the state-of-the-science test that now offers unsurpassed sensitivity for virus disease diagnosis. In this test, an enzyme to DNA first converts the viral dsRNA. Using another enzyme, the DNA then is copied more than a million times, similar to using a photocopier. The large quantity of the copied DNA can be detected easily in the laboratory, even though the original viral RNA from which it was copied may have been present in the mushroom tissue at levels too low to detect by other methods (Figure 38). Using RT-PCR, virus testing can be extremely sensitive, and few virus episodes escape undetected. The test is of reasonable cost and does not require extraordinarily expensive lab equipment.

Control Measures

Since there is no known commercial mushroom strain that is resistant to virus disease, the grower must incorporate virus disease preventative measures into the IPM plan and rigorously carry out the control measures. The following practices are recommended for disease control:

● Establish and strictly adhere to a complete sanitation/hygiene program. Sanitation of surfaces, machinery, and clothing of workers is the foundation of the control program. Disinfectants (quaternary ammonia solutions, iodine, phenolics, and chlorine) can be used in the cleaning regimes.

● Control the release and movement of spores to prevent them from infecting new crops. Do not allow mushrooms on the beds to open; pick tight. Pick diseased crops last.

Use HEPA air filters on production room air intakes.

- Attend to composting and pasteurization. Adequate and uniform peak heats are necessary to kill sources of infection in the compost (virus-infected spores and mycelium). Check routinely to ensure pasteurization with good peak heating during Phase II.

- Be careful to avoid contamination during spawning operations, and be protective of spawned beds. Practice thorough sanitation during spawning operations. Movement of workers should be restricted in the area where spawning operations are carried out and where spawn run crops are growing. Use plastic sheets on beds during spawn run to prevent spores from falling on the compost.

- Time-released supplementation at spawning may improve yields from a diseased crop, but is not a substitute for any of the control measures listed here. It cannot control the disease.

- Steam off to kill sources of infection such as mushroom spores and mycelium. Usually 160°F (71°C) in the beds for 6 hours or more is effective. Some growers use a 24- to 48-hour steam-off. Double steaming the house, once when full and again when empty, has been practiced but probably is not any more effective than steaming the house properly once.

The Future

Considerable success has been achieved in genetically engineering viral resistance in plants, and work is under way to accomplish the same for mushrooms. The first highly efficient and convenient procedure for transferring genes into *Agaricus bisporus* recently was developed (C. P. Romaine laboratory). Through molecular biotechnology, the breeding of viral-resistant mushroom strains, as well as strains with wide-ranging novel traits, now is within our grasp.

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