Verticillium Dry Bubble

This informational document is designed to be a constructive review of Verticillium Dry Bubble, the most common fungal disease of the commercial mushroom Agaricus bisporus.

Figure 1. Mushroom infected with Verticillium dry bubble showing the mass of tissue symptoms.

Understanding more about the biology of the fungus that causes dry bubble may help growers control this disease. For some growers the disease is just a re-occurring nightmare. With the difficulty in obtaining new or maintaining previous pesticide registrations, it appears that the struggle with this disease will continue for many years. It is the goal of this fact sheet is to provide growers with some basic biology and practical information about this disease.

Symptoms

Various symptoms for this disease have been observed. The manifestation of these diverse symptoms is due to when infection occurs during a stage in mushroom development and the quantity of spores that cause the initial infection. The stage of development at which infection occurs, how many spores start the infection and knowing the time from spore to symptoms, all appear to influence the type of symptom we might find. Therefore, recognizing all symptoms will help to determine when the initial infection occurred and that information can be used to control this disease. Several good papers describing the relationship of spores and disease development have been written (Sinden, 1971; Gandy, 1973; North and Wuest, 1993).

Bubble

The most conspicuous symptom found is described as a globe-like mass of mushroom tissue, a dry bubble, Figure 1. A single mushroom or a cluster of mushrooms can become a spherical mass of infected tissue covering an entire infected area. Occasionally as the diseased tissue ages, a few amber drops of liquid may form. These liquid drops often cause confusion with another fungal disease, wet bubble, caused by *Mycogene sp*., Figure 2. However, the difference is that only a few infected mushrooms exhibit the amber drops and for those that do, it is often only a few very small drops. Wet bubble is characterized by large and numerous amber drops covering the mass of mushroom tissue. The bubble symptom usually indicates an early and severe infection of the mushroom pin or even before the pin is visible. The early infection disrupts the growth of the mushrooms tissue preventing it from developing into differential shapes of the stem and cap.

Figure 2. Mushrooms infected with *Mycogene sp.* displaying the wet bubble symptom.

Split Stipe

When infection takes place after the pin begins to develop, and stem is infected, the stem splits as it matures causing a symptom described at split stipe or stem blowout, Figure 3. The infection disrupts stem elongation on one side of the mushroom, while the healthy side continues to grow normally. The tissue on the infected side shatters, splits or ruptures causing this characteristic symptom.

Figure 3. Mushroom infected with *Verticillium* dry bubble showing split stem symptom.

Necrotic Spots

This symptom is described as large brown spots with a fuzzy grayish hue in the center, Figure 4. These spots are usually the diameter of a pencil eraser head to a dime size. The fuzzy grayish growth covering these spots is the sporulating fungus and surrounding dark brown color helps distinguish it from bacterial blotch disease that is a lighter, more yellowish brown color. This symptom usually develops when infection occurred later in the mushroom growth, when pins are larger or a small spot infection occurred on a pinhead surface.

Figure 4. Mushroom infected with *Verticillium* dry bubble necrotic spot symptom.

Spotting

A needle pinhead size brown spotting of the mushroom cap is another symptom. These spots are often confused with spotting from other fungi such as *Trichoderma* or sometimes bacteria pitting, Figure 5. This symptom most likely occurs when a small spore load is present and infection occurs later in the pinning process.

Figure 5. Mushroom infected with *Verticillium* dry bubble with spotting symptom.

Symptomless

Some researchers have reported that mushrooms can be symptomless on the bed before or right after harvesting, and spotting develops later. These mushrooms may not be salable by the time they reach the store shelves, but more importantly, they may act to spread spores around the farm. Harvesters would unknowingly touch infected mushrooms and move spores to uninfested areas.

Causal Organism

Pathogen (organism causing the disease): *Verticillium fungicola* (Preuss) Hassebrauk var. *funigicola*

This fungus that causes the disease dry bubble, *Verticillium*, is also a pathogen on many wild mushrooms. Therefore, it is commonly encountered around the farms during the warmer weather when other wild fungi and molds grow, Figure 6. The easiest method to identify this pathogen is to culture it on selective media. This selective media was developed to allow *Verticillium* to outgrow any competitors. Details on how to prepare the media are Rinker et al., 1993. When *Verticillium* is grown on potato-dextrose agar (PDA), at room temperature, the colonies are white and the underside of the plates is colorless to yellow. Some cultures may have a low profile with rounded, scalloped edges. If one can measure the spores under a microscope, they would be about 8-10 mm long by 1-3 mm wide and tapering to 0.5-1.0 mm at the tip. The important morphological characteristic from a dissemination and disease control perspective is that the spores are produced in a gelatinous matrix. This gummy material will collect and hold many spores together. This sticky material is also what attaches the spores to flies, people and equipment. This gelatinous mass of spores is easily attracted by water particles, making anything to do with water especially susceptible to spreading the spores around a farm.

The fungus produces a tremendous quantity of microscopic spores, which cannot be seen by the naked eye. It has been estimated that an infected mushroom could conservatively produce 30 million spores within an hour. It has also been reported to take only 4,500 spores per lb. of casing to cause a partial loss and 45 million spores per lb. of casing to get no mushrooms. The spores and mycelium of the fungus may persist in a dry condition for up to six months and can survive the winter months inside a farm or under covered debris around a farm. Controlling the spread of this disease is like fighting an invisible enemy.

Disease Development

Early Infestation

The earliest infestation by the *Verticillium* spores may take place as early as casing time, but not before. Generally spores that land on the spawned compost will not cause a disease problem. It has been suggested that even spores that are on the compost before casing do not cause disease development. However, it has been recently shown that very high quantities of spores applied to the compost before casing can induced disease development (Beyer and Kremser, unpublished). Once the casing is applied and rhizomorphs begin to develop, then it seems that the *Verticillium* spores become viable and aggressive around the mushroom spawn. Spores that locate on the casing and spawn in the casing will germinate and disease will develop.

Secondary Spread by Vectors

The spores of dry bubble will spread within a production house or from crop to crop. One easy and most common method of spore dispersal is with water. It has been demonstrated that 60 drops of water on a single location splashed spores a distance of 2 feet (Cross and Jacobs, 1969). Therefore, watering on top of an infected mushroom located along the sideboard would splash spores onto watering personnel. Unknowingly, that water person would then spread the spores along the bed and to other crops they watered that day.

Spores are oftern carried on dust particles and water droplets in the air. Large water droplets are not necessary to carry spores. A fine mist, barely detectable, would be enough to move the spores. Dust particles act as vectors in much the same way, as the spores will stick to them, and if disturbed off the floors, they can land on the casing and infect the pins. Water vapor mist can transport spores rapidly around a room or farm. The washing of a floor, although a sound hygienic practice, may splash spores off the floor and onto the bottom beds or trays. Disease epidemics have been known to be

caused by excessive washing of the floors in the production houses. The rooms and farm look immaculate because growers will spend many hours washing floors with a hose or high pressure cleaning system. Unfortunately during this cleaning, spray from the floors carried *Verticillium* spores onto the beds and created a serious disease problem. This does not suggest that rooms should be kept dirty, but that cleaning should be done with a low-pressure water system or with no water at all. A squeegee is the best tool for cleaning up debris in a room because brooms will stir up dust particles that carry spores.

Flies are carriers of the disease and by controlling their populations, the occurrence of *Verticillium* can be reduced. Sometimes the disease might develop more quickly with an infestation of nematodes. This does not suggest that nematodes spread dry bubble but red spider mites, often found with high nematode populations, will pick up spores and move them around the bed or room. Other mites that feed on weed molds in the compost can be a problem. Eliminating the source of the food for these insects has many benefits. The mushroom will have more food available, less competition, and movers of spores will be eliminated.

Growers can also spread the disease. Every time they enter a house with dry bubble, spores are picked up and can be transported to a new crop. Therefore, growers should be conscious of their movement around the farm to minimize the dispersal of spores. Workers who monitor temperature and those who cover infected mushrooms with salt or alcohol are also potential carriers of the disease. North and Wuest (1993) have emphasized the importance of “symptomless” mushrooms on the development of this disease. Not only are mushrooms that show obvious symptoms potential sources of spores; some mushrooms that do not develop symptoms until after harvest can also be a spore source. These “symptomless” mushrooms can carry spores that can be spread by growers, harvesters and other personnel.

Dry bubble can also be a problem in the colder weather when mice invade a farm. Mice travel between rooms digging into beds to feed on the spawn grains. They run around on the floor picking up spores and often travel from old to new crops.

RH, temperature and other environmental factors

Environmental conditions within rooms will affect rate of disease development. The *Verticillium* fungus likes warm and moist conditions for growth and reproduction. It may be possible that higher humidity and temperature are responsible for the faster development and spread of the fungus in the summer. From the time a spore germinates to the time the symptom develops on the mushrooms was always thought to be about 14 days. Hybrid strains are generally grown at slightly warmer temperatures and more emphasis is placed on maintaining higher relative humidity for quality. Therefore, the disease development time is probably much shorter, perhaps 7-10 days. With a more favorable environment, the life cycle of the *Verticillium* is faster which means there will be more spores produced in a shorter time. More spores reduce spore to symptom development time. Therefore, depending on the amount of disease, we may consider a slight compromise to the environment in the room to slow the development of *Verticillium*.

It has been reported that a manipulation of the crop environment may reduce the amount of disease development. A reduction in air temperature from 68°F (20°C) to 57°F (14°C) and relative humidity from 90 to 80% for several days decreased the percentage of infected mushrooms (Nair and Macauley, 1986). A lowering of these parameters may slow the time of the spore germination or mycelial growth of the pathogen, and has less influence on the growth of the mushroom. However, in practice this manipulation of the environment can only be accomplished if the timing of the harvest is not adversely affected. It is also important to have adequate control of these changes to insure other diseases, like bacterial blotch, do not become a problem.

Control

Disease Monitoring

The first step in control is monitoring the crop to determine when the first bubble appeared. The timing of fungicide applications and locating the initial source of infection can only be done by accurately determining when the first symptoms are seen. Harvesters should be trained to know what diseased mushrooms look like and should alert the grower when the first symptomatic mushrooms are found. Some farms have harvesters place a stick or straw near the bubble, being extremely careful not to touch the diseased mushroom or the mushrooms around it. Using this marking system, the grower will know when the disease is found and then the salting or disease control crews can find the diseased mushrooms for treatment.

Unfortunately, this disease can rapidly escalate into an epidemic and then it takes weeks to gradually reduce the spore load on the farm to get the disease back under control. This reduction in the spore load around a farm and in the houses is a very important component of control. How to reduce this spore load is the problem. First, the vector(s) that is spreading

the spores must be identified and stopped. From the time of first symptom development the grower must look back to who or what was in contact with the crop about 10-14 days earlier; with warmer room temperatures or higher humidity, that time is shortened to about 7-10 days. Most often, dry bubbles spotted before the end of first break indicate that the crop was infested by something other than harvesting personnel or their equipment.

Chemical

The crop can be protected with any available fungicides or other techniques. It is not necessary to use fungicides until after casing. The registered fungicides available in the United States are Benlate (Benomyl), Bravo (Chlorothalonil) and Mertect (Thiabendazole). Bravo may be used in states where it is has a temporary EPA section 18 registration. Each year it is harder to renew this temporary registration in each state. The prospect of registering Prochloraz-manganese complex (Sporgon) or Mancozeb (Dithane M-45) appears bleak and, even if it is possible, it would many years, at a high cost, to complete the registration of these or any new fungicides. When fungicides are used, it is suggested to rotate or alternate their application. The rotation of fungicides may help to slow build up of resistance by *Verticillium* to one single product. Timing of a fungicide application is critical and therefore it is another important reason to determine accurately when you are first seeing symptoms. It is important to insure a complete coverage of the fungicide at the proper concentration. These products are expensive and therefore the applicator must make sure they reach into the middle of bottom beds as well as the back corners and around the uprights.

When using a disinfectant or chlorine bath to dip hoses or tools it is important to maintain the chemical's effective concentration. Dirt and organic matter in the tank decrease the effective concentration of the active ingredient. Any movement of watering or other personnel from old to new rooms, even for coffee breaks or to find a supervisor for instruction, must be stopped.

Cultural Control

Several cultural practices will affect disease development. It has been suggested that more spawn growth on the casing surface at the time of pinning relates to an increase the incidence of disease. This phenomenon would not suggest that a grower flush a crop earlier than normal to control the disease, because one may end up with an excessive number of dirty mushrooms. However, avoiding excessive overgrowth of the spawn on the surface may help lessen the disease. Furthermore, easier pinning strains may show less susceptibility than strains that are harder to pin because more vegetative spawn growth occurs on the surface than when "harder to pin" strains are used.

It cannot be emphasized enough, **keep the farm clean**. Not only does it instill a good mentality with the employees, it eliminates breeding grounds for *Verticillium*. During warm and moist weather organic matter and mushroom debris provide an excellent habitat for *Verticillium* to grow and rapidly produce spores. Therefore, spores are not only produced in infected crops, but additional spores are generated all around the farm.

Figure 7. Pile of salt covering an infected *Verticillium* dry bubble.

Many farms have one or more persons assigned to search out infected mushrooms and cover them with salt (Figure 7) or spray them with alcohol. This technique is more effective in the early breaks. Do not touch or remove the infected bubble. Heavily infected breaks are difficult to cover and it is usually too late to be effective. Since the spore load is so high in those rooms, the additional traffic around those crops may spread more disease. Unfortunately covering mushrooms that show symptoms does not prevent the spread of spores from "symptomless" infected mushrooms or other sources of infection. This covering or trashing of the bubbles should be done daily and be 4-6 inches in diameter, completely covering and surrounding the bubble. Table or rock salt, powdered 15% HTH or 80% alcohol can be used to cover or spray infected areas.

Watering hoses, harvesting equipment, flies, mites, harvesters, mice, watering personnel and growers are the obvious means of transportation for the spores that spread the disease. All pieces of equipment that come into a room or in contact with the crop after casing must be thoroughly cleaned and disinfected. If possible, for new crops or in each room, a separate water hose should be used. Dip hoses in a disinfectant bath between rooms if they have to be moved.

Other important procedures include insuring the floor of the room reaches 140°F for several hours during the peak heat. Often the compost temperature reaches 140°F before the floor or coldest area of the room reaches that temperature long enough to insure an adequate kill of carry over spores. After the spawning and casing operations, the floors should be cleaned to remove all bits of compost and casing and then sprayed with a strong disinfectant or chlorine solution. It is important not to get these chemicals on the compost or casing surfaces.

All tools and other equipment that are taken into the house anytime from spawning through to post crop steaming should be thoroughly disinfected. All harvesting baskets should be steamed or dipped in a disinfectant after each use and before they go back into the room for more harvesting. Spores of *Verticillium* and other diseases can be readily carried back into the houses on these baskets. No chemical should be used on these baskets, unless thoroughly rinsed off, to insure that no chemical residue ends up on the mushrooms.

Integrated Pest Management (IPM)

Improving other cultural problems on a farm will help reduce the spread of the spores. Proper composting and Phase II procedures will reduce weed molds and nematodes, sources of food for mites. Keeping the fly population under control is a critical part of control. Sometimes we "bite the bullet" and steam off heavily infested crops after second break. This "short term pain for a long term gain" is only effective if all other aspects of a control program keep the spores from re-infecting new crops.

Biological

To date the biological control products introduced into the mushroom industry have failed to adequately control this disease. The difficulty in using biological control agents for this disease may be in part to the inability of the biocontrol agent to become firmly established in the crop before the *Verticillium* fungus is present. Often, biocontrol agents require a certain amount of the pathogen to be active so that the control organism can survive. Preliminary results from some recent research have shown that processed spent mushroom substrate (SMS) used as a casing material suppresses disease development. The procedure for preparing the material as a casing has not been commercially developed and the amount of disease suppression was not economically feasible to use the process. Further research is being conducted to identify the suppression mechanism(s) which might be active in the SMS. However, some biological control products are helpful in controlling flies, which are a common vector of the disease. These products will be discussed in future information publications.

Conclusion/Summary

The scientist's goal is to learn more about the fungus. We need to know more about the nutritional and environmental requirements of *Verticillium* and the development of this disease. We know that manipulating the environment will affect disease progress, but doing it and maintaining the crop cycle and mushroom quality is difficult. Are there other cultural practices that encourage or discourage the growth of *Verticillium*? Are there biological control agents or compounds that we can use against it? Studying this disease must become a priority for the continued success of mushroom growing.

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