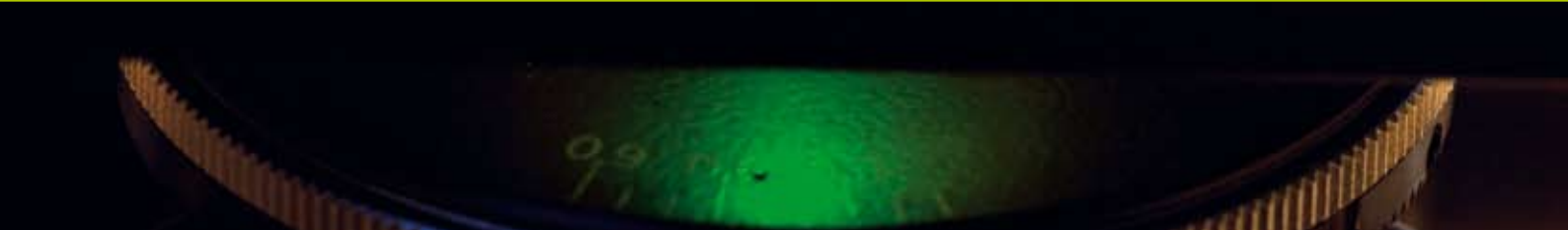
Microscope aids in discovering mechanism of action

Damaged **cell walls**



Fungi have a long history of ruining exquisite grapes. As a result, vineyards are the site of an ongoing battle against mildew and other fungal diseases. One agent used in this fight is iprovalicarb, which effectively inhibits fungi in the oomycete family. But how does it work? An ingenious experiment and a simple microscope helped Bayer find the answer: the fungicide attacks the cell walls.

Even in the era of molecular biology and genetic research, the microscope (left) still provides useful service to Bayer scientists in the development of agents to combat fungal diseases that infest vines (above).

Winegrowers have long known that a good wine is not made in the press, but in the vineyard itself. There are no hard and fast rules on how to proceed, however. The quality of the grapes is determined by a wide variety of factors. Age and type of vine, location, soil, precipitation, humidity and temperature all play a role. Above all else, winegrowers need to have a good deal of experience and creativity.

Sometimes, however, even all of those qualities are not enough. Capricious weather, insects, weeds and in particular fungi can easily put an abrupt end to good ideas for special wines. Grapevines can live 20 years or more, meaning that they often infect each other with diseases. In the autumn, the fungal spores fall to the ground with the leaves, only to re-infect the vine and its shoots in spring through wind and rain. If the winegrower does not quickly take counteractive measures, fungal growth spurred on by unfavorable weather conditions can easily destroy the entire harvest. Instead of distinctive, high-quality wine, growers then find themselves pressing rotgut.

Unfortunately, winegrowers cannot tell in advance which fungi might threaten their vines each season. Warm, humid days foster the growth of downy mildew, for instance, while dry weather encourages the powdery variety. Winegrowers are not alone in their fight, however. Researchers at Bayer CropScience have come to their aid with iprovalicarb, a fungicide specifically designed to combat fungi in the oomycete family, such as downy mildew.

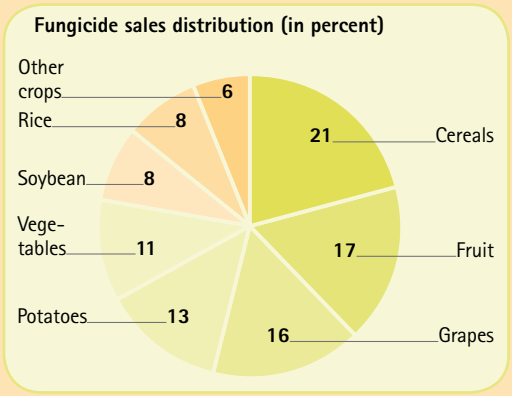
But how does iprovalicarb actually work? Until recently, no one knew the answer. The question is relevant because once the mechanism of action or the target molecule (which is attacked by the agent) are known, Bayer researchers can tailor the development of future active ingredients to these factors. Only when fungicides work according to different biochemical principles can winegrowers combat even those fungi which have become resistant to one specific agent.

Known mechanisms of action were ruled out

In order to answer the question, Bayer researchers first narrowed the range of possibilities. They tested the mechanisms and targets which had already been identified for other active ingredients. In the process, they discovered that iprovalicarb neither restricts the

The fungus Phytophthora can be easily bred in petri dishes in the laboratory (above). In this experiment, Dr. Andreas Mehl uses the microscope to monitor how iprovalicarb disrupts cell wall formation (light green, on the screen) (right).

Where fungicides are used



Fungal plant diseases can destroy entire harvests. Fungicides are therefore an essential part of agriculture. Products worth EUR 6 billion are used to combat harmful fungi around the world, one third of which in North and Latin America, a quarter in Asia and 42 percent in Europe.

mobility of reproductive cells – which move forward with the help of a flagellum in oomycetes, similar to sperm cells in mammals – nor impairs the respiratory chain to block the cell's source of energy. But they were still lacking a key hypothesis to explain how the fungicide works.

Dr. Isolde Häuser-Hahn from Bayer CropScience in Monheim and her colleague Dr. Andreas Mehl took on the task of solving this problem. After examining the facts at length, Mehl shifted the focus of his investigation away from the downy mildew Plasmopara viticola to the pathogen for late blight, Phytophthora infestans. In contrast to many other oomycetes, which as parasitic organisms are dependent on their hosts, Phytophthora can be cultivated in a nutrient solution. However, Phytophthora "is not easy to examine in a laboratory," reveals the agrobiologist.

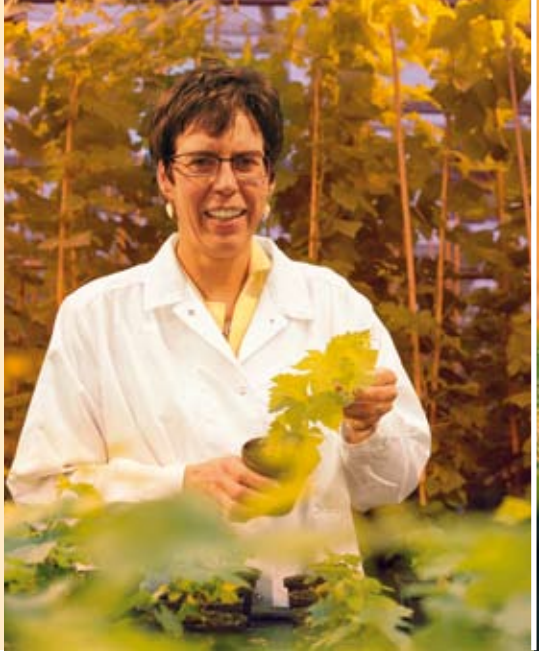
Biochemical tests were unsuccessful

Once Phytophthora was finally flourishing, Mehl took a look at the issue from a biochemical perspective and attempted to find a point in the fungi's metabolic activity at which iprovalicarb took effect. This produced no results. He also quickly exhausted the possibilities offered by the molecular biology methods which have now become an integral part of modern medical research. Even two-dimensional separation of the protein components according to isoelectric point and size failed to identify the site of action.

The researchers then decided to start over from scratch: iprovalicarb attacks only oomycetes. It occurred to Mehl and Häuser-Hahn that the main difference between this phylum and other groups of fungi is the structure of the cell wall. While the cell walls of downy mildew and similar fungi are stabilized with cellulose, other types of fungi rely on the harder and more robust chitin of which insect shells are made. The key to iprovalicarb's specificity for oomycetes could lie in damage to the cell wall structure. Unfortunately, little is known about the synthesis of cell walls in Phytophthora. "The scientific literature contains lots of information on cell wall formation in yeasts and plants, but very little on oomycetes," says Mehl.

An enzyme analysis initially failed to confirm this hypothesis. Glucan synthases – enzymes involved in the construction of the cell wall – displayed no

Dr. Isolde Häuser-Hahn (right) is tracking down the mechanism of action of iprovalicarb. This fungicidal active ingredient combats downy mildew (Plasmopara viticola), which can cause major damage to vines and triggers the typical discoloration of infested leaves (far right).



difference in activity levels when exposed to iprovalicarb. But Mehl was not about to give up. Instead, he dug deeper into his bag of laboratory tricks to design further tests. He created a cocktail of various enzymes to digest the cell wall. Individual round cells formed, enclosed in nothing more than a layer of cell membrane. Mehl then placed these protoplasts in a special nutrient solution, enabling the individual cells to begin rebuilding a cell wall. After this process was finished, he turned to an instrument which quietly occupies a place of honor among the industrial devices such as mass spectrometers, pipetting machines and gene sequencers typically found in modern laboratories – the microscope.

Microscope studies led to the breakthrough

Using a special cellulose stain which causes fungal cells to glow with uniform intensity, Mehl was able to record the correct rebuilding of the cell wall over time. When a precisely measured dose of iprovalicarb was applied, enough to impair the growth of the fungi but not completely hamper it, patterns appeared: stripes, knobs, bulges and blotches. That was the breakthrough. These patterns were evidence of the fact that iprovalicarb inhibits correct cell wall growth. In order to explore iprovalicarb's detailed mechanism of action, however, more research needs to be conducted on the actual process of cell wall formation. "No one knows exactly which enzymes, structural proteins and cofactors are involved in the process in fungi or oomycetes," says Isolde Häuser-Hahn.

A number of scientists at university botanical institutes are sure to be interested in examining the details of cell wall formation. These difficult studies with oomycetes do require a great deal of meticulous care, but that is precisely what makes them an exciting quest for creative fungi researchers.

www.winedirectory.org
Private U.S. website with over 8,000 links to information on wine

Fact file on iprovalicarb

Bayer CropScience launched its new fungicide iprovalicarb on the international market under the product name Melody® Duo in 1998 and then on the German market as Melody® Multi in 2001. Iprovalicarb specifically combats a group of fungi known as oomycetes such as the downy mildew Plasmopara viticola, which infects grapevines, and the pathogen for late blight, Phytophthora infestans. The latter was responsible for the failure of the potato crop in Ireland for several years in a row in the mid-19th century, ultimately reducing the country's population by half. Iprovalicarb prevents and combats fungal infections as well as impairing spore formation. A member of the new amino-acid-amide-carbamate class of active substances, it also helps combat oomycete strains which have become resistant to other fungicides. Iprovalicarb is not toxic to other classes of fungi – and therefore causes no damage to the very welcome wine yeast on the grape skins – or to insects, birds, fish and mammals. The active ingredient dissolves in water and soil within a just a few weeks' time, thoroughly penetrates the plant and is also transported into the new shoots.