ACARISIN™

In Botrytis Cinerea growth inhibition

Efficacy Study

Overview

The fungus belongs to the *Sclerotiniaceae* within the *Ascomycetes.* The anamorph stage Botrytis cinerea has a worldwide distribution. However, it is not clear how widespread and frequent the teleomorph B. fuckeliana is. It is concluded that sexual reproduction might be an important source of genetic variation.
The fungus grows well on artificial media. The anatomy and morphology of the mycelium of B. fuckeliana are typical of the Ascomycota. Septa are present and are perforated by a simple pore. The mycelium is composed of brownish olive hyphae. Anastomoses between hyphae are often noted. Conidiophores are frequently 2 mm or more long, mostly 16-30 µm wide with often a swollen basal cell. The conidia are ellipsoidal or ovoid, generally with a slightly protuberant hilum. They are dry and hydrophobic, colorless to pale brown, smooth, 6-18 x 4-11 µm (mostly 8-14 x 6-9 µm). In mass the conidia are gray-brown.

Biology

Damage

Gray mold is a blight or rot of immature, fleshy or senescent tissues. Lesions develop as tan or brown, water-soaked areas, which may become grayish or dried out. The profuse gray-brown sporulation of the fungus on old diseased tissue is characteristic. Buds and young shoots of grapevine may be infected, turn brown, and dry out. On a few leaves reddish brown, necrotic lesions appear on the vines. Before cap fall, the fungus may invade inflorescences, which rot and fall off. From these sites it attacks the pedicel or the rachis. From ripening onward, the grapes are infected, especially through wounds. The mold progressively invades the entire cluster. Infected white grapes turn brown, and black grapes become reddish.

Lifecycle

Spore germination is greatly stimulated by low concentrations of simple sugars, such as glucose and fructose, as well as by amino acids. A 0.015-0.02 µm narrow, blunt infection peg may form directly from the tip of the germ tube or from the appressorium or from each of the hyphal tips in the infection cushion in contact with the cuticle, which it penetrates directly. The penetration takes place directly through the surface of the organ partly by a mechanical process and partly by a chemical process involving cutin-degradating and pectin-hydrolyzing enzymes. B. cinerea does not usually penetrate through stomata but infection via wounds is common for this species. Epidemics caused by B. fuckeliana occur in cool, wet and humid weather, conditions that favor sporulation and infection. The severity of gray mold is closely related to environmental conditions and is especially dependent on temperature and relative humidity. The infection of grapes by the fungus requires at least 25 h at 15-20°C and saturated relative humidity.

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Cause

Under adverse conditions sclerotia are generally considered to be the most important structure involved in the survival. They recovere from the soil in vineyards and can survive long enough to serve as a source of inoculum for the following season. They consist of a medulla and a dark cortical layer of cells.
B. cinerea probably survives winters in the sclerotial stage. The production, size and shape of sclerotia on natural substrata and in culture are extremely variable. Sclerotia may germinate producing mycelium, conidiophores and conidia or apothecia. B. cinerea may also produce microconidia (phialospores). Microconidia enclosed in a protective coat have apparently only a sexual function as spermatia for the spermatization of sclerotia. They appear after long periods of unfavorable growth conditions. The coat becomes slimy in water and is able to stick to vectors. Apothecia produced from sclerotia range from 3 to 10 mm (up to 25 mm) high and from 1 to 6 mm diameter. Ascospores are uniseriate, one-celled, ovoid or ellipsoid, measuring 11.5-17.5 x 4.5-9.5 µm (av. 13.9 x 7.0 µm) in water. Chlamydospores of B. cinerea are hyaline single cells of extremely variable form and size. They are formed under conditions unfavorable for growth as terminal or intercalary cells by transformation of vegetative mycelium parts and are liberated by hyphae disintegration.

Conidia of B. cinerea are dry and easily dispersed in air currents. They are released by a hygroscopic mechanism and so are more abundant in the air when rapid changes of relative humidity occur during the day. The role of wind-blown and rain-splashed plant debris containing mycelia as dispersal propagules is probably underestimated.

Occurrence

Wide range of major and minor host plants. Citrus, tomato, Strawberry, Cranberry, Grape, Orange, etc.

Agricultural Importance

Botrytis bunch rot or gray mold exists in all vineyards in the world. Bunch rot seriously reduces quality and quantity of the crop. In some cultivars and under certain climatic conditions in autumn, infection of grape clusters takes on a particular form known as 'noble rot'.
Grape cultivars differ in susceptibility based on the compactness of bunches, thickness and anatomy of the berry skin, and their chemical composition. No major resistance genes against the fungus are known, although some intrinsic resistance mechanisms occur, such as the production of stilbene compounds in grapevine. The strategy of breeding for resistance is also difficult due to the broad genetic plasticity of the fungus.

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Control

Useful non-chemical contribution to Integrated Weed Management
Cultural practices must accompany other control methods in order to limit the damage caused by gray mold. Generally they aim at reducing the inoculum and determining environmental conditions that are unfavorable to infection.
Biological control is not used extensively; antagonists like the saprophytic fungus Ulocladium atrum and the mycoparasites Trichoderma spp. are described.

Chemical Control
Fungicidal control of B. cinerea is widely practiced all over the world. Several effective fungicide classes are available. In table-grapes effective pre-harvest control reduces inoculum and the number of infection sites on fruits and vegetables and is, therefore, crucial to prevent gray mold development during storage and transport. Control of post-harvest infection in cold-stored table grapes can be achieved by repeated fumigation with sulphur dioxide or by the use of sulphur dioxide generating pads closed in polyethylene-lined grape containers.

Beside in table-grapes, the control of Botrytis is also a standard measure in most wine-producing regions. Especially in years or regions with wet weather conditions two to three applications are often needed for good Botrytis control although major fluctuations in disease intensity are usually seen over several years. Typically, the Botryticide applications are made at mid to late flowering, before bunch closure and before harvest. Contrary to most multi-site fungicides, tolylfluanid, a sulphamide compound, shows considerable efficacy against Botrytis and is therefore used alone or in combination with specific botryticides.

Until the 1990ties dicarboximide fungicides such as iprodione have been used intensively for the control of Botrytis in grapes and many other fruit and vegetable cultures. Upcoming resistance to this fungicide class has reduced the effectiveness somewhat. However, as dicarboximide-resistant Botryris isolates show a significantly lower fitness than fully sensitive ones, a reduced number of dicarboximide applications gives still satisfactory results in many situations. Resistance management has become more easy with the introduction of three highly active fungicide classes in the 1990ties:

The anilinopyrimidines with pyrimethanil as effective representative, the phenylpyrrols and the hydroxyanilides with the highly specific botryticide fenhexamid as the only representative. More recently, a carboxamide fungicide showing also activity against Botrytis has been introduced. The simultaneous use of more than one mode of action in a season has clearly improved the overall Botrytis control und decreased the importance and frequency of resistant Botrytis isolates.

We have studied in-vitro natural eco-friendly control for spores through our wide-known best-sell product Acarisin™ Powder, that shows spectacular results @ 10g/L dosage conforming a promissory control system and product for B. cinerea in an Eco-Firendly Non-Toxic Production of Crops.

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In-vitro Efficacy Study against *Botrytis cinerea*

@ Peruvian National Agrarian University of La Molina, within the Department of Phytopathology and Nematology Clinic a Study was performed in 2018 with the following results:

1.- Materials and method

For evalutation of efficacy the poisoned food method was used on a culture PDA (Papa Dextrose Agar) on 4 Petri plates by treatment with a Control plate. Plates were inoculated with a 7 mm. disc obtained from a fresh colony of fungus on observation. Incubation temperature of inoculated plates was 24 ºC for a period of 7 days.

Treatments are shown within the Table 1. Dosage was 10g/L of Eco-Eff™ Control plate has no treatment.

Table 1

Treatment	Doslfication	Pathogen
1. Acarisin	10g/L	B.cinerea
2. Acarisin	n/a	B.cinerea

2.- Results

Results are shown in Table 2 and image 1.
We observed that Acarisin™ @ 10g/L inhibited 100% mycelia growth of Botrytis cinerea where in-vitro conditions in a PDA culture.

Table 2. mean of colony growth an percentage of control of B. cinerea in poisoned food of Acarisin™product at dosage 10 o/oo in a PDA culture.

(1) PC Commercial Product

Image 1 shown % of inhibition rate (figure 1) and colony diameter (in mm.) of Botrytis cinerea (figure 2) in poisoned foodstuff of Acarisin™ @ 1o 0/00 in PDA culture.

Table 2

PRODUCT	DOSIS (o/oo) PC[1]	COLONY DIAMETER (mm) final	% Control
Acarisin	10.00	0	100
CONTROL	0.00	85	0

Image 1 figure 1

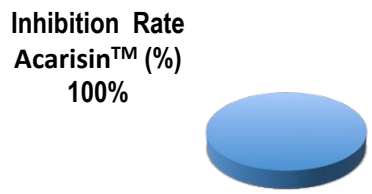
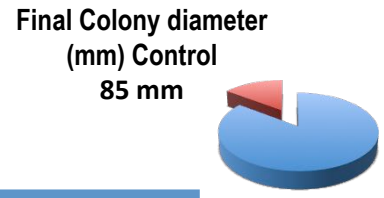


Image 1 figure 2



3.- Conclusions

Acarisin™ inhibited @ 100% mycelia growth of Botrytis cinerea when at in-vitro conditions in a PDA culture.

Product	Dosis (o/oo) PC	Diameter of colonies (mm)/Number of days since inoculation of 7mm plate											
		2				3				6			
		R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
Acarisin	10.00	0	0	0	0	0	0	0	0	0	0	0	0
Control	0.00	27	31	29	29	58	59	61	63	85	85	85	85

R1,R2,R3 and R4 are repetitions and/or plates. PC is commercial product. Dosage of 0.00 for Control plates.

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