Identification and Response to Fungicides of *Colletotrichum gloeosporioides*, Incitant of Strawberry Black Rot in Italy

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ABSTRACT

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The agent responsible for a black fruit rot of strawberry in Italy was identified as *Colletotrichum gloeosporioides*. The influence of temperature and pH on the growth of the pathogen is reported. Among the fungicides tested, prochloraz, followed by captafol, chlorothalonil, captan, dichlofluanid, thiram, and tolclofos-methyl, gave the best results in vitro.

During the spring of 1983, about 40% of the ripening strawberries (*Fragaria vesca* L.) were lost to an unusual fruit rot disease in some greenhouses in Northern Italy. Infected berries developed brown to black, slightly sunken, circular lesions about 3 mm in diameter that rapidly enlarged within 2–3 days to involve most of the fruit. Around the margin of the lesion, the mycelium of a fungus grew out, giving it a fuzzy appearance. In the center of the spot, masses of pink to brown conidia were easily seen. During hot, dry weather, the spots appeared deeply sunken and the masses of conidia were pink, becoming almost orange, on ripe fruit. The disease is called *marciume nero delle fragole* (strawberry black rot). All cultivars of strawberries grown in the area (Gorella, Belrubi, and Humigento) were equally susceptible to the disease.

The aim of this work was to isolate and identify the agent of the disease and to study some of its biological characteristics and its sensitivity to several fungicides to aid in the development of field control strategies.

MATERIALS AND METHODS

A fungus belonging to the genus *Colletotrichum* was easily isolated on potato-dextrose agar (PDA) from the infected fruit. The fungus grew on PDA as circular white colonies that darkened to gray and after a few days contained salmon-colored conidiophores and conidia. The conidia measured 13×4 nm. R. A. Samson (Centraalbureau voor Schimmelcultures, Baarn) confirmed our identification as *Colletotrichum gloeosporioides* Penz. (*Glomerella cingulata* (Stonem.) Spauld. & Schrenk) (8). The symptoms of the disease were easily reproduced by inoculating fruit either wounded (with a sterile pin) or not wounded, both attached to plants (cultivar Belrubi) under greenhouse conditions and detached (cultivars Gorella and Belrubi) under laboratory conditions. Inoculations with mycelial disks and conidial suspensions (10^6 conidia per milliliter) were successful. The pathogen could be reisolated from artificially infected fruit.

Table 1. Mycelial growth of *Colletotrichum gloeosporioides* on different solid media after 7 days of growth at 22 C

Medium	Growth (mm)
Carrot agar	53.0
Czapek agar	32.5
Malt agar	53.5
Fresh PDA	52.5
V-8 juice agar	62.5
PDA (Merck)	51.5

Anemone (*Anemone coronaria* L.), which in Northern Italy is severely attacked by a *Colletotrichum* sp. (2), was inoculated with the isolates from strawberry by dipping corms in conidial suspensions (10^6 conidia per milliliter) for 10 min. After artificial inoculation, corms were transplanted in steam-disinfected soil. All experiments were carried out using two isolates of the pathogen obtained from two strawberry varieties (Gorella and Humigento). Hyphal growth of the pathogen in petri dishes 90 mm in diameter was compared on six solid media (carrot agar, Czapek agar [Merck, Darmstadt, West Germany D-6100], malt agar [Merck], PDA [Merck], freshly prepared PDA, and V-8 juice agar) after 7 days incubation at 22 C (Table 1).

The effect of pH, ranging from 4 to 8, and of temperatures, ranging from 5 to 45 C, on the growth of the fungus was studied by transferring 4-mm mycelial disks onto malt agar and measuring the radial hyphal growth after 6 and 10 days of incubation.

The effectiveness of several fungicides, as technical compounds (Table 2) belonging to different chemical groups known for their activity against *Colletotrichum* or other strawberry pathogens, was tested in vitro on PDA in 90-mm-diameter petri dishes. Mycelial disks of the pathogen (7 mm in diameter) were transferred onto increasing concentrations of the different chemicals, which were added to the medium as 100-fold concentrated methanolic suspensions. Only iprodione was dissolved in acetone

Table 2. Effectiveness of different fungicides against colony formation and mycelial growth of *Colletotrichum gloeosporioides*

Fungicides	ED_{50} (ppm)[a]		MIC (ppm)[b]	
	Colony formation	Mycelial growth	Colony formation	Mycelial growth
Benomyl	>100.0	0.3	>100.0	1,000
Captafol	<0.1	15.0	1.0	>1,000
Captan	1.0	100.0	5.0	>1,000
Chlorothalonil	0.2	3.0	0.3	>100
Dichlofluanid	2.0	30.0	3.0	>100
Dicloran	20.0	10.0	30.0	>100
Etaconazole	30.0	0.3	100.0	10
Iprodione	>1,000.0	3.0	>1,000.0	>1,000
Mancozeb	5.0	100.0	10.0	300
Prochloraz	0.2	<0.1	0.3	3
Thiram	3.0	2.0	10.0	>1,000
Tolclofos-methyl	5.0	3.0	10.0	100
Vinclozolin	>1,000.0	50.0	>1,000.0	>1,000

[a] Effective dose, 50%.
[b] Minimal inhibitory concentration.

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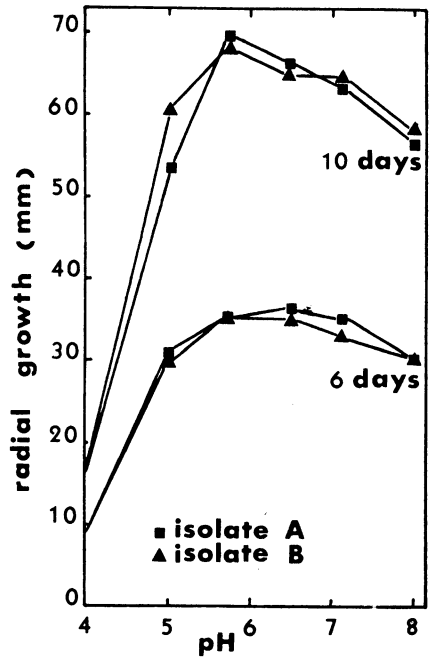

Fig. 1. Effect of pH on mycelial growth of *Colletotrichum gloeosporioides* on malt agar after 7 days of incubation at 22 C.

because of its known rearrangement in alcoholic solution (1). Radial hyphal growth was measured after 7 days of incubation at 22 C. Conidial suspensions of the pathogen (10^3 conidia per milliliter) were distributed (100 μl/plate) into petri dishes over the surface of PDA containing increasing concentrations of the different fungicides, and the number of developing colonies was counted after 3 days of incubation at 22 C and compared with the number of colonies developing on control plates. Results are expressed as effective dose, 50% (ED_{50}), and as minimal inhibitory concentration (MIC). All data are the means of at least three replicates for each of the two isolates; all experiments were repeated at least twice.

RESULTS

C. gloeosporioides grew best on V-8 agar, followed by malt agar, carrot agar, fresh PDA, and PDA (Merck). On Czapek agar, the growth rate was significantly lower (Table 1). On malt agar, the fungus grew better at pH values ranging between 5 and 7 with an optimum at 5.7 (Fig. 1) and at temperatures between 17 and 25 C with an optimum at 22 C (Fig. 2).

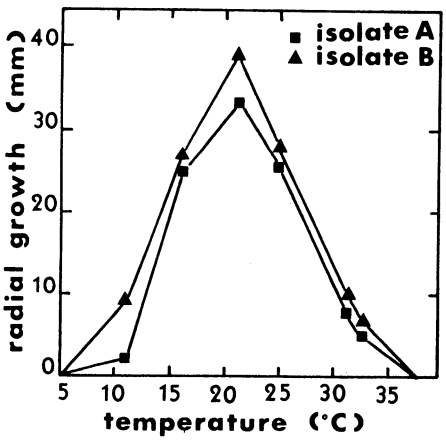

Fig. 2. Effect of temperature on mycelial growth of *Colletotrichum gloeosporioides* on malt agar after 7 days of incubation.

Among the tested fungicides, prochloraz was the most effective, showing a strong inhibition, at low dosage, of both mycelial growth and colony development. Captafol, chlorothalonil, captan, dichlofluanid, thiram, and tolclofos-methyl showed good activity. Benomyl was ineffective (high MIC), but the low ED_{50} against mycelial growth cannot be explained. Dicarboximides (iprodione and vinclozolin) were completely ineffective; iprodione showed a low ED_{50} against mycelial growth but had a very high MIC value (Table 2).

Artificial inoculation of anemone corms with the isolates from strawberry resulted in the appearance of leaf curling, and the isolates from anemone inoculated on detached, wounded strawberry fruit produced symptoms.

DISCUSSION

Strawberry rot caused by *C. gloeosporioides* was observed in Italy for the first time, apparently because of the unusually favorable conditions (warm and high relative humidity caused by persistent rain) existing during the spring of 1983. The very poor growth shown by the pathogen at temperatures below 10 C indicates that the fungus can cause practical problems only during warm weather conditions. The conidia of the pathogen, profusely produced under favorable conditions, are easily dispersed by splashing water.

A similar rot of strawberry fruit was reported as incited by *Gloeosporium* sp. in Louisiana (9) and by *C. acutatum* in Australia (5,6), in Florida (4), and in Arkansas (7). *C. gloeosporioides* strains causing rot of strawberry fruit appear morphologically similar to those of the same species causing leaf curling of anemone in the same area (2). The sensitivity of the two pathogens (from strawberry and from anemone) to the different fungicides was similar: in both cases captan, captafol, chlorothalonil, and dichlofluanid showed good activity, whereas benzimidazoles and dicarboximides were ineffective. Only tolclofos-methyl, active against *C. gloeosporioides* from strawberry, was ineffective against the same species from anemone (3).

The complete ineffectiveness of dicarboximides, which are almost the only fungicides used to control gray mold (*Botrytis cinerea*) of strawberry, at least partially explain the easy development and spread of the pathogen in the area.

From a practical point of view, use of such fungicides as captafol, captan, or dichlofluanid alternated or mixed with dicarboximides should be suggested; in this way, it should be possible to control *B. cinerea* and *C. gloeosporioides* and to delay the risk of appearance of strains of *B. cinerea* resistant to dicarboximides.

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