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The Relationships between Fungicidal Action on *Diaporthe citri* and Effect on Mitochondrial Respiration of 8-Quinolinol and Its Related Compounds

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INTRODUCTION

Melanose, a rain-borne disease of citrus caused by *Diaporthe citri* (Faw.) Wolf,¹⁾ is prevalent in Japan which has an abundant rainfall during the growing season of citrus. The fungus also causes stem-end rot in stored fruit and pycnidia are formed on the dead twigs of citrus trees.²⁾ The pycnosporos are carried on leaves or fruit by raindrops, germinate, form appressoria and penetrate the tissues. After penetration, the fungus dies and the fruit does not become corrupt,³⁾ but reddish brown to dark brown spots caused on the surface of leaves or fruit form tear-streaked or mud-caked pattern in case of heavy infection and lower the grade of fruit because of the appearance. Therefore, melanose is one of the most important diseases of citrus in Japan. Copper 8-quinolinolate, a chelate compound of 1 molecule of copper ion and 2 molecules of 8-quinolinol, has strong inhibitory effect against *D. citri*. It does not cause rash on the skin as zineb or mancozeb does and the toxicity to mammals is low.⁴⁻⁶⁾ Moreover, it does not cause phytotoxicity as inorganic copper compounds do.

In the previous paper,⁷⁾ a possibility was suggested that the inhibitory effect of 8-quinolinol on the growth of *Alternaria kikuchiana* is related to the uncoupling effect and 8-quinolinol acts as a carrier of copper ion to afford the synergistic fungicidal action of

copper ion and 8-quinolinol, because mitochondrial membrane or some related biomembrane is permeable to uncouplers. In this paper, we studied the relationship between the inhibitory effects of some compounds related to 8-quinolinol on the growth of *D. citri* and their effects on respiration of rat liver mitochondria, in order to clarify the mode of action of 8-quinolinol on *D. citri*.

MATERIALS AND METHODS

Spore-hornes of pycnidia produced on an autoclaved citrus twig culture of *D. citri* were collected and suspended in sterile distilled water containing 2×10^{-2} M fructose to give an appropriate density (100-120 pycnosporos in a field of $150 \times$ microscope magnification). This was then mixed with the same volume of solutions of quinoline, 2-,4-,5-,6-,7- and 8-quinolinols (3-quinolinol was unavailable), α -naphthol and 5,7-dihalogenated-8-quinolinols. EDTA (final concentration; 10^{-6} M) was added to solutions of 8-quinolinol or 5,7-dihalogenated-8-quinolinols in order to eliminate trace metals contained in the solutions.⁸⁻¹⁰⁾ Drops of the suspensions (pH 6, adjusted by 13 mM phosphate buffer) were placed on alcohol-treated epidermal strips of onion bulb and incubated at 25°C.¹¹⁾ After 24 hr incubation, pycnospore germination rate and hyphal penetration rate were observed under a microscope.

Effects of compounds relative to 8-quinol-

linol on respiration of rat liver mitochondria were observed with a Clark type platinum oxygen electrode. The suspension medium contained 0.2 M sucrose, 2 mM MgCl_2 , 1 mM EDTA·2Na, 10 mM succinate·2 Na and 10 mM K-phosphate buffer (pH 7.4). The medium also contained 1 μg of rotenone per mg of protein, in order to inhibit the electron transport system except for the succinate system.

RESULTS

I_{50} of compounds relative to 8-quinolinol on pycnospore germination of *D. citri* is shown in Table 1. Inhibitory effect of these compounds was in the order of, quinoline $\approx$ quinolinol $<$ α -

naphthol $<$ 5,7-dihalogenated-8-quinolinol. I_{50} of these compounds on hyphal penetration was about half of that on pycnospore germination.

Then, we studied the effect of these compounds on the respiration of rat liver mitochondria. In a series of experiments, 2-quinolinol had no influence on respiration even at 10^{-2} M and 4-quinolinol and 5-quinolinol slightly inhibited at 10^{-3} to 10^{-2} M (Fig. 1). Quinoline, 6-quinolinol, 7-quinolinol and 8-quinolinol accelerated respiration at 10^{-3} to 10^{-2} M (Fig. 1), α -naphthol accelerated at 10^{-4} to 10^{-3} M (Fig. 2), 5-chloro-7-bromo-8-quinolinol accelerated at 10^{-5} to 10^{-4} M and 5,7-dichloro-8-quinolinol and 5,7-dibromo-8-quinolinol also

Table 1 I_{50} of 8-quinolinol and its related compounds on germination of *Diaporthe citri*.

Compound	I_{50} (M)
Quinoline	3×10^{-3}
2-Quinolinol	3×10^{-3}
4-Quinolinol	7×10^{-3}
5-Quinolinol	5×10^{-3}
6-Quinolinol	1×10^{-3}
7-Quinolinol	3×10^{-3}
8-Quinolinol	1×10^{-3}
α -Naphthol	3×10^{-4}
5,7-Dichloro-8-quinolinol	5×10^{-5}
5-Chloro-7-bromo-8-quinolinol	3×10^{-5}
5,7-Dibromo-8-quinolinol	5×10^{-5}

Fig. 1 Influence of quinoline and quinolinols on oxygen consumption rate of rat liver mitochondria.

Q: quinoline, 2Q: 2-quinolinol, 4Q: 4-quinolinol, 5Q: 5-quinolinol, 6Q: 6-quinolinol, 7Q: 7-quinolinol, 8Q: 8-quinolinol.

Fig. 2 Influence of α -naphthol on oxygen consumption rate of rat liver mitochondria.

Fig. 3 Influence of 5,7-dihalogenated-8-quinolinols on oxygen consumption rate of rat liver mitochondria.

5,7diCl: 5,7-dichloro-8-quinolinol, 5Cl17Br: 5-chloro-7-bromo-8-quinolinol, 5,7diBr: 5,7-dibromo-8-quinolinol.

Fig. 4 Relationship between $\log 1/I_{50}$ and $\log 1/C_{1.5}$, where $C_{1.5}$ is the concentration that induces respiratory acceleration of rat liver mitochondria to 1.5 times.

Q: quinoline, 6Q-8Q: 6-quinolinol-8-quinolinol, α -N: α -naphthol, 5,7-diCl: 5,7-dichloro-8-quinolinol, 5Cl7Br: 5-chloro-7-bromo-8-quinolinol, 5,7-diBr: 5,7-dibromo-8-quinolinol.

accelerated at 10^{-5} to 2×10^{-4} M (Fig. 3). Relationship between inhibitory effect of compounds relative to 8-quinolinol on germination of *D. citri* and their effect on mitochondrial respiration is shown in Fig. 4. There was a close correlation between them ($n=8$, $r=0.944$). Inhibitory effect of these compounds on hyphal penetration of *D. citri* and their effect on mitochondrial respiration were also closely correlated, because I_{50} of all of these compounds on hyphal penetration was about half of that on pycnospore germination.

DISCUSSION

When no EDTA was added, 8-quinolinol revealed a two-peaked dosage-response curve as was shown in the case of *Stemphylium sarcinaeforme*.¹²⁾ It was not found on *Alternaria kikuchiana*.⁷⁾ When no EDTA was added, I_{50} of 8-quinolinol on germination of *D. citri* at lower concentration was $6 \times 10^{-7} \sim 2 \times 10^{-6}$ M and that at higher concentration was 10^{-3} M. Two-peaked dosage-response curve was also observed on the slide glass unless EDTA was added. Therefore, the inhibitory effect at lower concentration is considered to be due to chelate compound of 8-quinolinol and trace

metals contained in reagents or *D. citri* itself. On the other hand, 5,7-dihalogenated-8-quinolinol did not reveal two-peaked dosage-response curve even when no EDTA was added, that is, I_{50} of them was not influenced by EDTA. This might depend on the fact that chelate activity with trace metals was less than that of 8-quinolinol.

Quinoline, 6-quinolinol, 7-quinolinol, 8-quinolinol, α -naphthol, 5,7-dichloro-8-quinolinol, 5-chloro-7-bromo-8-quinolinol and 5,7-dibromo-8-quinolinol inhibited the growth of *D. citri*, i.e., hyphal penetration or pycnospore germination, at nearly the same concentrations as they accelerated mitochondrial respiration. Among the compounds tested, a close correlation was found between $\log 1/I_{50}$ and $\log 1/C_{1.5}$, where I_{50} is the concentration of 50% inhibition on germination of *D. citri* and $C_{1.5}$ is the concentration which induces respiratory acceleration of rat liver mitochondria to 1.5 times. Judging from this fact, the inhibitory effect of 8-quinolinol and the related compounds against *D. citri* seemed to be related to the uncoupling effect.

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要 約

8-Quinolinol およびその関連化合物のカンキツ黒点病菌に対する殺菌作用とミトコンドリアの呼吸に及ぼす作用との関係

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8-quinolinol およびその関連化合物の, カンキツ黒点病菌に及ぼす生育阻害作用と, ミトコンドリアの呼吸に及ぼす作用との関係を検討した. quinoline, 6-quinolinol, 7-quinolinol, 8-quinolinol は, $10^{-3} \sim 10^{-2}$ M でラット肝臓のミトコンドリアの呼吸を促進した. α -naph-

thol は $10^{-3} \sim 10^{-4}$ M, 5-chloro-7-bromo-8-quinolinol は $10^{-5} \sim 10^{-4}$ M, 5,7-dichloro-8-quinolinol および 5,7-dibromo-8-quinolinol は $10^{-5} \sim 2 \times 10^{-4}$ M で呼吸を促進した. これらの化合物は, ミトコンドリアの呼吸を促進する濃度とほぼ同じ濃度で, カンキツ黒点病菌の生育を阻害した. カンキツ黒点病菌の発芽を 50% 阻害する濃度を I_{50} , ラット肝臓のミトコンドリアの呼吸を 1.5 倍に促進する濃度を $C_{1.5}$ とすると, $\log 1/I_{50}$ と $\log 1/C_{1.5}$ との間には非常に高い相関関係が見られた ($n=8$, $r=0.944$).