The effects of Iprodione on the lipid metabolism of Botrytis cinerea.

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Botrytis cinerea is a fungus belonging to the class Ascomycetes, which includes some devastating plant pathogens. It causes grey mold of various important crop species such as oilseed rape, peas and beans. Iprodione is a fungicide used to control B. cinerea and belongs to the dicarboximide group of fungicides for which there is no known common mode of action. Iprodione has been reported to cause lipid peroxidation [1] but its mode of action is unclear [2-4].

The effects of Iprodione on the lipid metabolism of B. cinerea were determined by radiolabelled acetate feeding experiments in the presence and absence of Iprodione. The method of generating and harvesting mycelia and extracting lipid was generally the same for all experiments. B. cinerea spores were used to inoculate a flask of malt extract liquid media. Liquid cultures were incubated at 21°C, in the light for 24 hours. The cells were harvested and resuspended in fresh media and aliquoted into culture tubes.

Each aliquot was pre-incubated, for one hour with Iprodione, dissolved in dimethylsulphoxide (DMSO at 0.25% final concentration), the controls were incubated with DMSO alone. Cultures were incubated with 1µCi of [1-14C]acetate. After incubation, the cultures were filtered, washed and lipid was extracted using hot isopropanol as described by Kates[5]. Lipid classes were analysed by thin-layer chromatography and fatty acids by radio-gas-liquid chromatography.

In the first experiment the fungus was incubated for 4 and 24 hours in the presence of 10µM Iprodione. There was no change in uptake of radiolabel at 4 or 24h compared to control, but incorporation into lipid was reduced. At 24h there were changes in the proportions of olate and linoleate labelled and in lipid classes. Phospholipids (particularly phosphatidylycholine and phosphatidylethanolamine) were reduced while neutral lipids contained more radioactivity. Thus, labelling of diacylglycerols plus non-esterified fatty acids were increased 4.5-fold compared to controls.

In a time-course experiment, it was found that the inhibition of lipid labelling by Iprodione was continuous over a 24h period (Fig.1). With 10µM Iprodione, inhibition was about 20% at 2h increasing to 40% after 24h.

Increasing concentrations of Iprodione were used (Fig.2) and noticeable effects found between 1 and 10µM with regard to lipid class labelling at 24h for incubations continually in the presence of reagent. Above 10µM there was no further changes in the relative labelling of lipids (Fig.2). The most striking change was a decrease in the percentage labelling of polar lipids (mainly phospholipids) and a corresponding increase in diacylglycerol labelling. This result may indicate an effect of Iprodione on the final enzymes of the CDP-base pathway.

Growth inhibition of the fungus with 10µM Iprodione was determined by changes in dry weight and radial growth. After 24h the dry weight was 80% of the control and at 48h it was 73%. Radial growth was markedly affected with concentrations of Iprodione up to 10µM, above which there was no extra inhibition. It was interesting that these data agreed exactly with the concentration effects on lipid labelling (Fig.2) and emphasise the connection of lipid metabolism with the general growth and development of B. cinerea.

Iprodione affected the lipid metabolism of B. cinerea at concentrations which were inhibitory to growth. The most effective concentrations were 1-10µM. The main effect on lipid metabolism was a decrease in total lipid. Phospholipids were also reduced with a corresponding increase in diacylglycerols. Since diacylglycerols are precursors in the phospholipid biosynthetic pathway, it suggests that Iprodione is inhibiting reactions utilising diacylglycerols.

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