ANTIFUNGAL COMPOUNDS IN FRECKLE-INFECTED BANANAS AND THEIR ROLE IN ANTHRACNOSE DEVELOPMENT.

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EXTENDED ABSTRACT

Freckle disease of banana caused by *Phyllosticta musarum* is widespread in Sri Lanka. While the disease continues to adversely affect the banana export industry, the freckled fruits are quite accepted by the local consumer. The disease is characterized by pin-head sized, restricted and isolated spots in the outer tissue layers of the fruit peel. Freckles are rough to the touch and heavy infections may give a reddish-brown colour to the skin affecting the external appearance and lowering market quality (Abayasekara *et al* 1993). The infections that take place when the bunch is young do not expand into progressive rots during fruit ripening. The disease therefore does not result in quantitative losses. In contrast to freckle, the anthracnose disease (*Colletotrichum musae*) causes severe postharvest losses to ripe bananas in every banana growing region of the world (Waller, 1992). The disease is generally controlled by postharvest treatment with fungicides. In Sri Lanka too the anthracnose is the most damaging postharvest disease in banana, but no adequate control measures are practiced.

The incidence of anthracnose disease was observed to be significantly less in freckle-infected fruits var. '*Ambul*' (Mysoor, AAB group) than those without freckles. A negative correlation was observed between the density of freckle infections and the severity of anthracnose lesions that develop during fruit ripening. A similar trend was observed when unripe bananas having varied freckle spots were artificially inoculated with *C.musae* where significantly lesser anthracnose lesion development occurred in heavily freckled fruits than those with lesser freckles. However, the anthracnose fungus, *C.musae*, could be isolated more frequently from the surface-sterilized peel of freckled fruits than non-freckled fruits showing that more latent infections are found in the freckled fruit skin.

This investigation has further shown that the freckle infection is associated with the accumulation of at least three phytoalexins in the peel tissues of unripe bananas. Peel tissues were excised from freckled and non-freckled unripe banana fruits separately and extracted in ethyl acetate by vacuum infiltration for 4 hours. The extracts were concentrated and bioassayed on thin layer chromatography plates using conidia of *Cladosporium cladosporioides* or *C.musae*. The extracts obtained from freckled fruit peel produced three antifungal zones. The most prominent antifungal zone was at Rf 0.60, which was present in peels with low, moderate or high levels of freckling. The other two antifungal zones were observed only when freckling was moderate or high in the fruit peel, one being more prominent (Rf 0.31) than the other. In general the total antifungal activity was greater in the heavily freckled bananas than those with moderate or low freckle infection. The extracts of healthy banana peels did not show any antifungal activity. Freckle-infected fruit peels continued to show antifungal activity during the ripening process. The antifungal activity could be detected even in fruits that are infected by the freckle fungus at a very early stage of maturity. The results of the investigation indicate that the accumulation of antifungal substances in response to freckle infection may be a major factor that makes the banana fruits resistant to anthracnose disease. In order to chemically separate the antifungal compounds, a large scale extraction was carried out using 100 g of freckled peel tissue. The extract was fractionated by flash chromatography at the Department of Chemistry, the active fractions were identified by TLC-

bioassay. The purification of antifungal constituents from the active fractions is at present in progress.

This is the first record of accumulation of phytoalexins in response to freckle infection of banana fruit peel. Earlier Mulvana et al, (1968) showed that ethanolic extracts from healthy, green *Cavendish* banana were antifungal and suggested that this might restrict the growth of *C. musae in vivo.* However, Muirhead (1981) found no evidence of a preformed antifungal compound that restricts the growth of *C. musae*. He attributed the antifungal activity to oxidation products formed during extraction from the phenolic compound, dopamine. In another investigation unripe bananas were inoculated with conidia of *C. musae* and the necrotic spots resulted beneath the inoculum droplet, when bioassayed, contained five fungitoxic compounds. These compounds were not present in healthy tissues (Brown and Swinburne, 1980). The compounds produced in infected tissues apparently disappear with fruit ripening. There were no reports of any follow up work on these fungitoxic compounds.

Induction of resistance has been shown to be an effective way of protecting plants from pathogen attack (Wilson *et al* 1994). Non-pathogenic or less aggressive microorganisms and mild strains of pathogens have been used to induce resistance in fruits and vegetables against postharvest pathogens. Strains of *Penicillium funiculosum* non-pathogenic to pineapple have been used to protect the fruits from pathogenic strains of this fungus (Lim and Rohrbach 1980). The present investigations have clearly indicated that the freckling induces fruit resistance to anthracnose through accumulation of phytoalexins. Many Sri Lankans believe that the freckled banana fruits are more tastier than the non-freckled ones. Freckling appears to offer yet another advantage by naturally suppressing anthracnose disease. It is possible that the development of a strain of the freckle fungus or an elicitor, capable of inducing similar phytoalexin response without inflicting freckle symptoms, may help protect banana fruits from anthracnose.

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