A STUDY OF THE LATENT INFECTION OF IMMATURE AVOCADO (*PERSEA AMERICANA*) FRUIT BY *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ.

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ABSTRACT

This thesis consists of four chapters. A part of the first chapter carries the results of a survey conducted of avocado cultivation, harvesting, storage and marketing practices employed and the extent as well as the causes of post-harvest losses in this commodity. Second part of this chapter describes the anthracnose disease in avocado, its latent phase in particular and a few experiments performed to test for surface stimulants on avocado fruit of germination and infective structures formation by Colletotrichum gloeosporioides.

The major object of this study was however, to identify the physiological factor/s that control the latent infection development in immature avocado by Colletotrichum gloeosporioides Penz.; the factors considered were the antifungal activity of fruit tissue and the enzyme potential of the fungus to degrade the immature fruit cell wall. The second and third chapters which deal with these two aspects therefore carry bulk of this whole study. The fourth chapter contains the general discussion and conclusions.

The survey has shown that substantial fruit losses take place in almost every stage of fruit production chain. Improper harvesting techniques, inadequate storage facilities, lack of proper storage technique and rough handling of fruit at vertually every stage of the whole process being some of the causes for such losses. 70-80% of these losses were found to be due to microbial

spoilage. Five fungal diseases were found responsible for most of the microbial spoilage of which anthracnose disease accounted for about 50-60% loss.

Anthracnose disease (C. gloeosporioides) in ripe avocado originates as latent infections in the immature fruit. Conidia which germinated on immature fruit surface quickly produced hyaline appressoria which within another few hours turned into dark thick-walled structures. Further growth of the fungus did not take place until fruit ripening commenced. Immature fruit surface and its leachates were stimulatory to germination and appressoria formation by the fungus.

The fungus produced much higher levels of exo-polygalacturonase trans-eliminase and proteolytic enzyme a) in liquid cultures incorporated with Water Insoluble Components (WIC) from immature and ripe fruit, b) in autoclaved -immature fruit and c) in ripe fruits. The amount of pectolytic enzyme produced by C. gloeosporioides in liquid cultures with WIC of immature and ripe was almost similar, 42.5 and 43.0 units ml^{-1} respectively. The pH optima of exo-PGTE and protease were 8.5 and 7.5 respectively in all enzyme preparations. The rotted fruit tissue contained endo- polygalacturonase (endo-PG) which was found to be endogenous to avocado fruit had a pH optimum of 5.5. Both in vitro and in vivo enzymes macerated immature avocado fruit tissue discs in vitro within 3 hrs of incubation but did not do so with potato tuber discs. Neither immature nor ripe fruit contained inhibitors, proteinaceous or otherwise,

which can inhibit the activity of pectolytic or proteolytic enzyme production by C. gloeosporioides. It appeared that the enzyme potential of the pathogen to invade immature fruit cell wall is sufficient but some other factor seems to prevent the fungal development, hence the enzyme production.

Concentrated ether extract of the peel of healthy immature avocados when bioassayed on thin layer chromatographic plates with conidia of either Cladosporium cladosporioides or C. gloeosporioides, produced four inhibition areas at R_f 0.30, 0.32, 0.70 and 0.75(these were denoted as Av IV, Av III, Av II and Av I ,respectively). Inoculation of fruit with C. gloeosporioides did not increase their amounts suggesting that post-infectionally formed compounds are absent. A hot chloroform extract was partitioned on a silica gel column and the four antifungal compounds were separated. Spectroscopic data revealed that one of these compounds (Av II) was similar to cis-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (Prusky et al., 1982) and another (Av IV) was a long chain saturated compound comprising hydroxyl group(s) having molecular weight of 268. Toxicity of Av II to C. gloeosporioides was two times more than Av IV and seven and a half and six and a half timesmore than Av I and Av III respectively. The amount of these four antifungal compounds increased gradually during fruit development and reached their maximum at harvest. These were concentrated more in the deeper tissues of the fruit than in the superficial layers. The concentration of these compounds Av I, Av II, Av III and Av IV was 1300, 920, 1050 and 780 $\mu g\ g^{-1}$ fresh weight of peel respectively, in the fruit at harvesting maturity. The amount of Av II and Av IV decreased to 53 and 64 $\mu g\ g^{-1}$ fresh weight of peel

respectively at ripe fruit and no Av I and Av III was detected. This took place in coincidence with the onset of progressive lesion development by the fungus.

The results strongly established the fact that the presence of antifungal compounds was the most important factor controlling the latency of unripe avocado fruit to C. gloeosporioides and that their decline below toxic levels during ripening allows the fungus to develop progressive rotting.