

Abstract.

The aims of this project were twofold: (a) to compare the accumulation patterns of antifungal diene (Z,Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,-15-diene) and triene (Z,Z,E)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene) compounds in harvested and unharvested “Fuerte” avocado fruits in response to inoculation with *Colletotrichum gloeosporioides* and relate these patterns to observations of the infection cycle, and (b) to use rDNA ITS nucleotide sequence analysis to confirm the identities of some important fungal pathogens of avocado. One of these pathogens was an apparent complex of *C. gloeosporioides* and *Pseudocercospora* sp. (CgP complex). Inoculation of harvested and unharvested fruits with *C. gloeosporioides* at approximately 240 d after fruit set caused an increase in diene and triene levels as measured by HPLC and HPLC-MS compared with controls. In both experiments, these levels reached a maximum during the first 2 d, and subsequently decreased during the 7 d monitoring period. However, there were some notable differences in fluxes of these compounds between experiments. In harvested fruits during the active period, diene levels were higher in the flesh than the peel, whereas triene levels were higher in the peel than the flesh and triene levels decreased to below those of controls in the later stages whereas diene levels did not. Similar patterns emerged for unharvested fruits but diene levels in unharvested fruits were relatively higher than in harvested fruits whereas triene levels were similar in harvested and unharvested fruits. By employing SEM ellipsoid spores characteristic of *C. gloeosporioides* were first observed on the harvested inoculated fruit surfaces at 2 d, followed by surface hyphae at 3 d. However, the infection process of *C. gloeosporioides* in unharvested fruits was delayed, relative to harvested fruits, probably due to high antifungal diene and triene contents of unharvested

fruits. In contrast to SEM observations, ellipsoid spores of *C. gloeosporioides* were observed on the inoculated fruit surfaces at 7 d by using CLSM, and no surface hyphae were noted. The ITS universal primers were employed to confirm the identities of the fungal pathogens; Primer pair of ITS5 and ITS4 was used to amplify genomic rDNA from the CgP complex, *Pseudocercospora* sp. and *Phomopsis* sp., while ITS1 and ITS4 primers were used to amplify *Colletotrichum* sp. genomic DNA. Expected PCR product sizes of ~550 base pairs were obtained for all amplifications. Phylogenetic analysis revealed that the CgP sequences were closest to a *Phomopsis* sp., and confirmed the identity of *Pseudocercospora purpurea*, *Colletotrichum gloeosporioides* and *Phomopsis perseae*.