Abstract

The potential of the mycoparasite, Verticillium lecanii, at protecting citrus fruits against green mold was explored at the cellular level. Treatment of the fruit with V. lecanii or chitosan prior to inoculation with the causal agent of green mold, Penicillium digitatum, markedly reduced disease development compared with that of nontreated control citrus fruits in which symptoms were visible by 3 days after inoculation with the pathogen. Scanning electron microscope investigations of citrus samples, collected 5 days after inoculation with the pathogen, revealed striking differences in the extent of cell surface colonization between treated and nontreated fruits. Pathogen hyphae, which sporulated abundantly at the surface of control fruits, were collapsed and severely damaged in V. lecanii- and chitosan-treated fruits. Histological observations of citrus samples confirmed that restriction of pathogen colonization at the cell surface correlated with a pronounced disorganization of the pathogen hyphae. In addition, host cell changes, mainly characterized by the deposition of a new material in the exocarp cells and the thickening of cell walls, were observed. Ultrastructural investigations of citrus samples revealed that the pathogen multiplied abundantly through much of the mesocarp and exocarp tissues in V. lecanii-free citrus fruits, whereas in V. lecanii-treated citrus, pathogen growth was restricted. Penicillium hyphae that penetrated the mesocarp tissue were markedly altered. Labeling with the wheat germ agglutinin/ovomucoid-gold complex for the localization of chitin resulted in an irregular labeling of Penicillium cell walls, even at a time when these were markedly altered. Cytochemical investigations revealed that callose and lignin-like compounds accumulated at sites of pathogen colonization in the exocarp tissue. Evidence is provided in this study that V. lecanii as well as chitosan are equally capable of inducing a striking response in P. digitatum-infected citrus fruits. The marked differences observed in the rate and extent of colonization as well as in pathogen cell viability between control and treated citrus fruits demonstrate that both treatments have the ability to induce the transcriptional activation of defense genes leading to the accumulation of structural and biochemical compounds at strategic sites.