

## Abstract

Black-spot symptoms, caused by *Alternaria alternata*, developed in persimmon fruits during prolonged storage at  $-1^{\circ}\text{C}$ . A preharvest treatment with gibberellic acid (GA3) extended the storage life of the fruit by delaying both black-spot development and fruit softening. Conversely, treatment of persimmon fruits with paclobutrazol (PBZ), an inhibitor of gibberellin (GA) synthesis, enhanced black-spot development and fruit softening during storage. Production of endo-1,4-beta-glucanase (EC 3.2.1.4, EG) by *A. alternata* in culture and in the presence of cell walls from PBZ-treated fruits as the carbon source, was enhanced by 150% over production in the presence of cell walls from control fruits, whereas endoglucanase (EG) production in the presence of cell walls from GA3-treated fruits was reduced by 49% relative to controls. To determine the importance of EG in symptom development, *A. alternata* EG was purified from a culture-inducing medium. It had a molecular mass of 41 kDa, its optimal pH and temperature for activity were 5.5 and  $47^{\circ}\text{C}$ , respectively, and the pI was 4.3. Its  $K(m)$  and  $V(\text{max})$  were  $0.43\text{ mg ml}^{-1}$  and  $18\text{ }\mu\text{mol reducing groups minute per milligrams of protein}$ , respectively. The internal sequence of a 21-mer amino acid peptide from the purified EG showed 62% similarity and 38% identity to the EG-1 of *Trichoderma reesei* and of *T. longibrachiatum*. Purified EG induced black-spot symptoms on the fruit, similar to those caused by *A. alternata*, whereas boiled enzyme caused only pricking signs. Our results suggest that the black-spot symptoms caused by *A. alternata*, in persimmon, are related to the ability of the fungus to produce EG in developing lesions.