

Effects of *Erwinia amylovora* harpin on tobacco leaf cell membranes are related to leaf necrosis and electrolyte leakage and distinct from perturbations caused by inoculated *E. amylovora*

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We have characterized the membrane potential response of cells in tobacco leaves to externally applied or injected harpin from *Erwinia amylovora* (Ea321) and linked it to subsequent electrolyte leakage and eventual necrosis. We similarly examined depolarization in response to injected Ea321 cells, and mutant bacteria that are defective in harpin production. Injected and applied harpin depolarize only the H⁺-ATPase-dependent component of membrane potential, in contrast to injected wild-type Ea321, which greatly depolarizes both pump and diffusion potentials. The functional capacity of the H⁺-ATPase component is also much less impaired in harpin- than Ea321-injected leaves. Furthermore, harpin production does not account for the entire set of differences in response to injected wild-type and mutant bacteria. Membrane depolarization in response to applied harpin and necrosis induced by injected harpin are similarly concentration-dependent and directly related. Parallel inhibition of harpin-caused membrane depolarization, electrolyte leakage and necrosis by the Ca²⁺-channel blocker, La³⁺, or the protein kinase inhibitor, K252a, links these events and suggests that prolonged Ca²⁺-entry and phosphorylation underlie them. Although harpin similarly depolarizes leaf and suspension cell potentials, there is a substantial difference: leaf cell membranes are less sensitive and repolarize. Increased repolarization upon harpin removal points to probable reversible association with the plant cell. Dependence of repolarization upon order of harpin/fusicocin addition suggests separate modes or sites of action.