

Insect Feeding Mobilizes a Unique Plant Defense Protease that Disrupts the Peritrophic Matrix of Lepidopteran Larvae⁵

Tibor Pechan¹, Renuka Shivaji¹, Allen Cohen², W. Paul Williams³ and Dawn S. Luthé¹

¹Department of Biochemistry and Molecular Biology

²USDA-ARS Biological Control and Mass Rearing Research Unit

³USDA-ARS Corn Host Plant Resistance Laboratory

Mississippi State University, Mississippi State, MS 39759

We have shown that maize (*Zea mays* L.) lines resistant to feeding by fall armyworm (*Spodoptera frugiperda*) and other lepidopteran larvae, rapidly mobilize a unique 33-kD cysteine protease (Mir1-CP) at the wound site within 1 hour of larval feeding⁴. Accumulation of the Mir1-CP in the maize mid-whorl region was correlated with a significant reduction in larvae growth that was caused by impaired nutrient utilization. Black Mexican Sweetcorn (BMS) callus was transformed with *mir1*, the gene encoding Mir1-CP. When fall armyworm larvae were reared on BMS that expressed Mir1-CP, their growth was reduced by 60 to 80%. Scanning electron microscopy indicated that the peritrophic matrix (PM) was severely damaged when larvae fed on transgenic BMS or resistant plants. Damage to the PM probably impairs the insect's highly organized digestive system and may account for the reduction in larval weight.

We are currently investigating the cause of the rapid accumulation of Mir1-CP. RT-PCR using gene-specific primers did not reveal a significant increase in *mir1* mRNA in response to larval feeding, or treatment with methyl-jasmonate (JA), ethylene, or JA + ethylene. Treatment with 1-methyl cyclopropene, which blocks ethylene receptors, decreased *mir1* mRNA levels, prevented the accumulation of Mir1-CP and increased susceptibility to insect feeding. The rapidity of the Mir1-CP appearance and lack of increased mRNA abundance in response to larval feeding suggests that Mir1-CP accumulation is post-transcriptionally regulated. PSORT bioinformatic analysis predicts that Mir1-CP is an extra-cellular protein. Mir1-CP is glycosylated and contains a 15 amino acid sequence in the C-terminal region that has 53% identity with citrus tatter leaf tatter virus cell-to-cell movement protein. In wounded plants, immuno-gold localization indicated that Mir1-CP was in the plasmodesmata connecting phloem parenchyma and bundle sheath cells and in cell walls between sieve tubes. This suggests that Mir1-CP may move to the wound site in response to a signal elicited by larval feeding or wounding.

⁴Pechan, T. et al. 2000. A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other lepidoptera. *Plant Cell* 12, 1031-1040.

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