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Morphological Study of a Novel Cysteine Protease Effect On Lepidopteran Larval Peritrophic Matrix

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We are studying a chitin-binding, extracellular 33-KD cysteine protease (Mir1-CP) that rapidly accumulates in the whorls of maize inbred lines that are resistant to feeding by fall armyworm (*Spodoptera frugiperda,* FAW) and other Lepidoptera [1]. When fall armyworm feed on plant tissues containing Mir1-CP, their growth is retarded approximately 50-80% [2]. This growth reduction is due to impaired nutrient utilization. We examined the insect's first line of food defense, the peritrophic matrix (PM). The PM is a membrane-like structure that surrounds the food bolus, protects the insect mid-gut from chemical and physical damage, and plays an active role in digestion and nutrient absorption.

We have shown that the peritrophic matrix (PM) of FAW larvae that feed on plant material expressing Mir1-CP was damaged and treatment of isolated PMs with purified recombinant Mir1-CP increased PM permeability in a concentration dependent manner [3]. Dose response bioassay showed a decreased relative growth rate for FAW larvae fed on artificial diet containing concentrations greater than 600 ng/ml Mir1-CP (unpublished data, Mohan et al.,). This study was conducted to morphologically determine the *in vivo* damage of the PM in fall armyworm larval species by Mir1-CP.

Although no visible midgut difference was observed between fourth instar control and Mir1-CP treated larvae (Figure 1), distinct morphological PM damage was observed by light microscopy study for larvae fed with greater than 600 ng/ml Mir1-CP (Figure 2). Damaged fall armyworm larval PM under similar Mir1-CP treatment conditions was also observed by scanning and transmission electron microscopy techniques (Figure 3, 4). These results show a unique maize defense proteinase (Mir1-CP) being responsible for reducing fall armyworm larval growth by morphologically damaging their peritrophic membrane.

References

[1] T. Pechan et al., The Pl. Cell 12 (2000) 1031-1040.
[2] B. Jiang et al., Pl Physiol 108 (1995) 1631-1640.
[3] S. Mohan et al., J. Insect Physiol 52 (2006) 21-28.

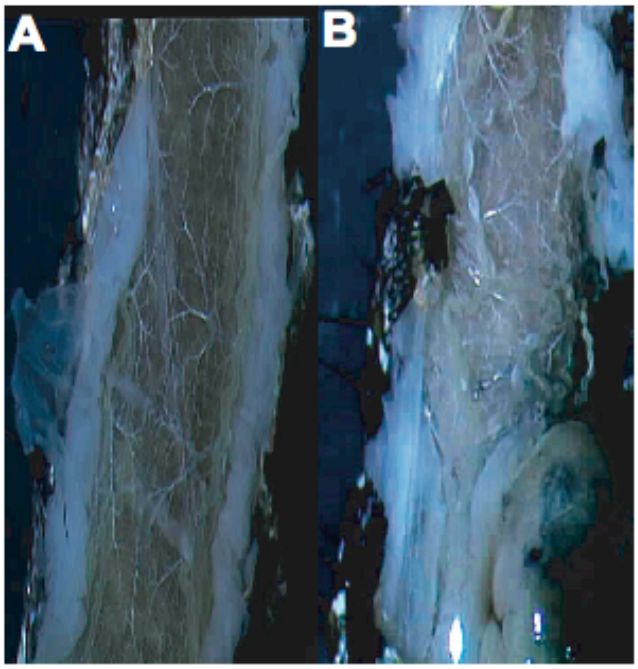

Figure 1. Visual comparison of midgut morphology. (A) Midgut of 4th instar FAW feeding on normal artificial diet. (B) Midgut of 4th instar FAW feeding on artificial diet containing > 600 ng/ml of Mir1-CP. Visual comparison pictures were photographed at 1x dissection scope magnification

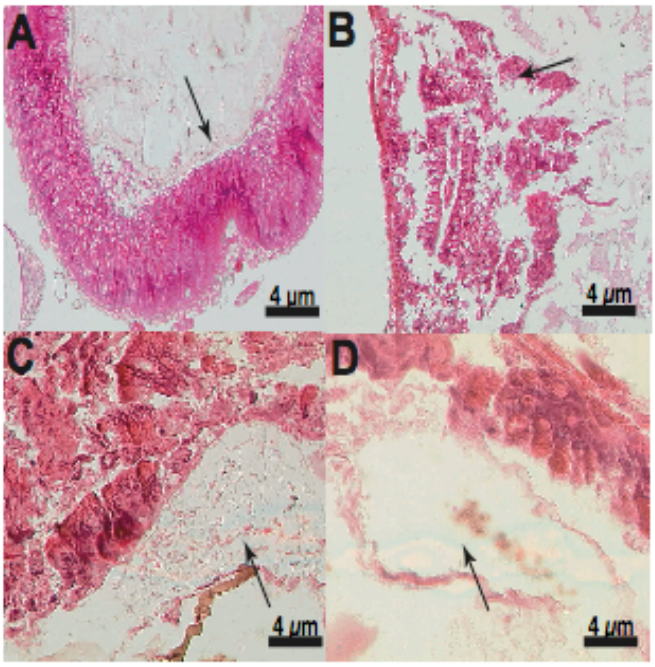

Figure 2. microscopic analysis of Mir1-CP effect on FAW PM (A) Cross secton of healthy larval midgut PM. (B) shearing of the midgut integrity when fed on artificial diet containing 60 µg/ml Mir1-CP. (C) and (D) shows PM disintegration when fed on 6 µg/ml and 0.6 µg/ml Mir1-CP, respectively. Arrows indicate the PM condition under each treatment. Results were based on three independent trials observed at 10X magnification.

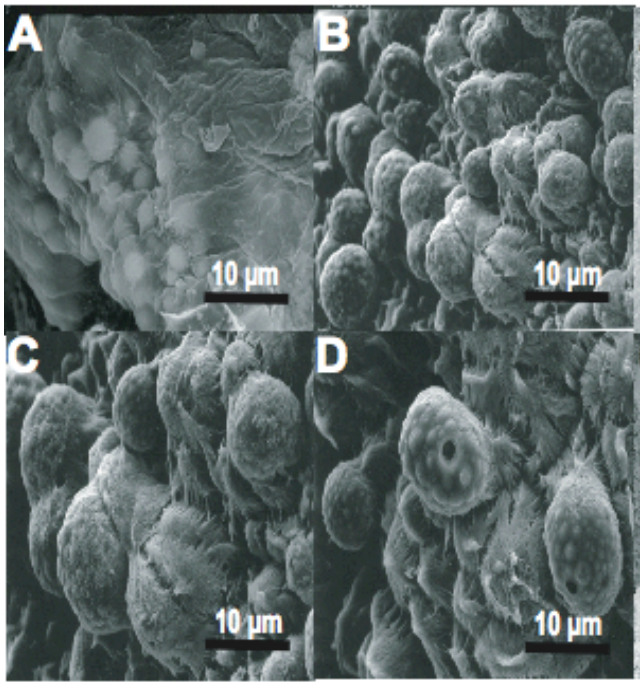

Figure 3. Scanning electron microscopy study of Mir1-CP effect on FAW PM. (A) Control PM at 700X magnification. (B) PM treated with Mir1-CP of > 600 ng/ml at 700X magnification. (C) and (D)SEM images under similar Mir1-CP treatment conditions observed at 1400X magnification.

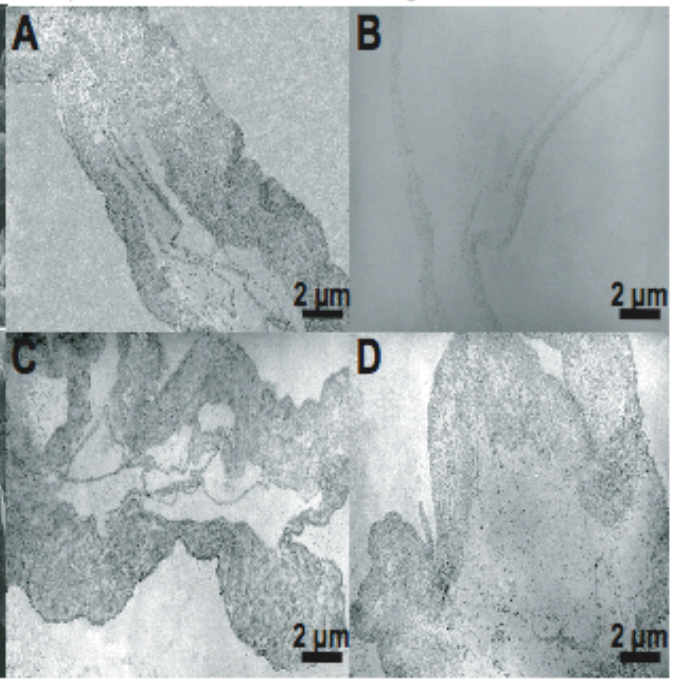

Figure 4. Transmission electron microscopy study of Mir1-CP effect on FAW PM. (A) Control PM . (B)PM treated with 600 µg/ml of Mir1-CP. (C) and (D) PM treated with 60 and 0.6 µg/ml Mir1-CP, respectively. All micrographs were documented at 5000X scope magnification.

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