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Abstract

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Towards RNAi-mediated pest mite management: Ingestion, cellular uptake, and intracellular processing of long dsRNAs*

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Double-stranded RNA (dsRNA) as a trigger of RNAi-mediated sequence-specific gene silencing is a promising next-generation pesticide that could enable selective pest control ultimately at the species level. The discovery that orally administrated dsRNA induces RNAi in the two-spotted mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), has led to a dramatic increase in research toward the development of RNAi-based biopesticides for spider mites. We have developed a nylon mesh-based simple feeding device for efficient oral ingestion of dsRNA by mites. This device enables highly efficient screening of essential genes in which lethal RNAi is induced by orally administrated dsRNA, and several candidate targets have been found in *T. urticae*. In addition, we found that adult *T. urticae* females could ingest particles smaller than about 0.5 μm using their stylets, providing insight into the design of carriers that could serve as effective delivery and protection of dsRNA. We also found that orally ingested macromolecules such as dsRNA localize within digestive cells floating in the lumen of the midgut in *T. urticae*, suggesting that the cells are a promising target for RNAi. Furthermore, measuring the activity of dsRNA dicing, the first process after the uptake of dsRNA by the cells, revealed the involvement of two *Dicer* genes. These results as well as the experimental systems constructed in this study will accelerate the development of RNAi-based biopesticides for spider mites and contribute to the management of potential RNAi resistance caused by mutations not only in target genes but also in RNAi machinery.

Keywords: Dicer, midgut, pesticides, stylets, *Tetranychus urticae*

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