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学位（博士）論文要旨  
(Doctoral thesis abstract)

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論文題目 Title	An effective method to screen dsRNA as a foliar-applied acaricide against the two-spotted spider mite, <i>Tetranychus urticae</i> Koch  (ナミハダニに対する葉面散布型殺ダニ剤としての応用を目的とした二本鎖RNAの効果的な選抜方法)				
論文要旨 (和文要旨(2000字程度)または英文要旨(500words)) ※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。 Write a summary in Japanese (2000 characters) or in English (500words). If the abstract is written in Japanese, needed to translate into English.  The two-spotted spider mite (TSSM), <i>Tetranychus urticae</i> , is a chelicerate arthropod distributed over the temperature zone and one the most important polyphagous pests. TSSM can attack over 1,100 different plant species and rapidly develops resistance to acaricides because of the short life cycle and the higher ability in detoxifying xenobiotics. In addition, easy maintenance for experimental populations, recently revealed TSSM genome and developed RNA interference (RNAi)-based reverse genetics protocols have been boosting this species as a model pest chelicerate particularly for studies of pesticide resistance and plant-herbivore interactions. TSSM's whole genome has been sequenced as the first complete chelicerate genome and is the most compact genome at 90 megabases (54% is protein coding sequence) among sequenced arthropod species. RNAi has a sequence-specific function of post-transcriptional gene silencing, which is triggered by double-stranded RNA (dsRNA) delivered into cytoplasm. Delivery of dsRNA to TSSM have been applied by 5 methods including injection of dsRNA into the mite body, mite soaking in a dsRNA solution, and mite feeding on dsRNA-containing artificial diet, dsRNA-infiltrated leaf discs or dsRNA-expressed transgenic plants. Among these methods, mite feeding on dsRNA-infiltrated leaf discs mimics foliar application of dsRNA as a sprayable pesticide. Previous studies showed that the rapid distribution of an aqueous solution of dsRNA over the upper					

surface of leaf discs was achieved by using an organosilicone surfactant (Silwet L-77). Then, dsRNA with Silwet L-77 would be infiltrated into mesophyll cells on which TSSM feeds and deliver to the midgut. This method allows researchers an efficient bioassay for the screen of dsRNA as a foliar-applied acaricide against TSSM. However, Silwet L-77 has known to possess toxicity to TSSM and this may be obstructive to evaluation of the effect of dsRNA. Here, I developed a novel means of preparing leaf discs in which a polypropylene sheet rather than a surfactant was used to ensure sufficient distribution of an aqueous solution of a tracer dye. These leaf discs were then successfully used to deliver the tracer dye into the midgut of TSSMs. In addition, no significant differences were observed in survival, fecundity, and feeding activity of TSSMs fed on leaf discs treated with this method compared with those fed on untreated leaf discs. Using this method, I delivered dsRNA of a gene encoding the membrane-associated vacuolar H<sup>+</sup>-ATPase (V-ATPase) which is an evolutionally-conserved enzyme presents in all eukaryotes. Down-regulation of the *V-ATPase* gene in TSSM (*TuVATP*) increased mortality and decreased fecundity as the dsRNA concentration increased 0.01 to 1 μg/μL. My method can eliminate any concerns for bioactivity of surfactants and thus is anticipated for a wide range of applications on oral delivery-based bioassays for the screen of dsRNA as a foliar-applied acaricide against TSSMs.

(英訳) ※和文要旨の場合(300 words)

If the abstract is written in Japanese, needed to translate into English.(300 words)