CONFOCAL MICROSCOPY CONFIRMS THE FEASIBILITY OF GENE SILENCING FOR EMERALD ASH BORER SUPPRESSION

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Emerald ash borer (EAB) (*Agrilus planipennis*) is an aggressive, invasive forest and urban tree pest that feeds and develops beneath bark, killing trees rapidly. We are investigating the use of RNA interference (RNAi) as a tool for managing EAB. Previously, we showed that oral ingestion of double-strand RNA (dsRNA) can silence essential and specific genes, and subsequently found in droplet assays that silencing heat shock protein and shibire genes kills up to 90 percent of neonates and adults after 1–2 weeks of exposure. To evaluate our findings in vivo, we assessed dsRNA uptake through plant material and through the egg chorion. Twigs of greenhouse-grown tropical ash, *Fraxinus uhdei*, were treated with labeled dsRNA and infested with EAB eggs; twigs treated with unlabeled dsRNA were used as controls. Confocal microscope images were then taken and analyzed to detect fluorescence in both plant and insect material. Additionally, EAB eggs were soaked in labeled dsRNA, and eggs and hatched neonates analyzed by confocal microscopy. Gene silencing was performed to corroborate imaging results. After 8 days of exposure, we detected fluorescence in ash bark and xylem, and larvae fed on these plants showed strong fluorescence inside their alimentary canal. When eggs were soaked with labeled dsRNA, fluorescence was detected in eggs and the hatched larvae, confirmed by gene expression. Our twig assay demonstrated that dsRNA is taken up through plant material, suggesting that trunk injections or soil treatments with dsRNA may potentially be an efficient delivery method for this technology. Absorption of dsRNA by eggs demonstrated that spray-able dsRNA may be a potential delivery method. EAB is rapidly expanding its geographic range, and innovative means of management are essential to mitigate its impacts. We've shown that RNAi in EAB is feasible; here we demonstrate the potential of topical applications of this technology.

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