Advances in RNA interference: dsRNA Treatment in Trees and Grapevines for Insect Pest Suppression

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RNA interference (RNAi) is a breakthrough technology that has significantly impacted contemporary approaches to control the damage caused by insect pests. The method permits functional genomics studies in many organisms that are difficult to study, moving science beyond model systems and laboratory studies. Reviews on applications of RNAi promise to improve human health and agricultural production (Castanotto and Rossi 2009, Siomi and Siomi 2009, Bellès 2010, Huvenne and Smagghe 2010). To move RNAi into real-world applications such as *Citrus* sp. and viticulture, we evaluated the movement and persistence of dsRNA in citrus trees and grapevines (Hunter et al. 2010ab). Most well-known RNAi studies continue to rely on injecting the dsRNA molecules directly into the organism; this approach is not suitable for use in the field. If host-delivered RNAi-based management approaches are to be implemented, plants must successfully uptake the dsRNA and retain it long enough for the target insects to ingest it through feeding (Hunter et al. 2010c, Huvenne and Smagghe 2010).

We propose the development of a new class of environmentally-friendly, ‘Highly Specific Pest Control’ (HiSPeC) substances. HiSPeC’s are highly specific, with no adverse effects on non-target species. Equally important, these substances are environmentally friendly and safe to handle. This report entails current efforts of HiSPeC’s that use plant-systemic delivery of an RNAi agent. To facilitate a real-world implementation of a host-delivered RNAi strategy, robust intake of the dsRNA and system-wide spread of the silencing action must be achieved. In some invertebrates, there exists a systemic RNAi pathway (Jose et al. 2009); other invertebrates depend on receptor-mediated endocytosis for cell internalization of the dsRNA (Saleh et al. 2006, Jose and Hunter 2007). The dsRNA can have very low LD-50, in the pictograms; results showed specificity to silence arginine kinase, AK-transcripts in psyllids and leafhoppers with the corresponding dsRNA experiments in lab tests. RNAi strategies will demand mass-production of dsRNA, efficient delivery methods, and methods to validate its environmental stability (Hunter et al. 2010c). The longevity of the dsRNA in *Citrus* trees showed suitability to develop an area-wide pest suppression approach. This study reports the robust nature and persistence of the RNAi pathway metabolites in whole-plant systems targeting insects. Persistence of dsRNA in psyllids and leafhoppers was detectable for 5-8 days post ingestion from plants, while detection in treated *Citrus* was at least 57 days post treatment (Fig. 1). Small interfering RNA (siRNA) was detectable into the third month.

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