“Synthesis of a full-length infectious cDNA clone for strain PN of Grapevine rupestris stem pitting-associated virus and the pathogenic characterization of 3 distinct GRSPaV isolates”

Grapevine viruses are nearly ubiquitous and can be responsible for up to 50% loss of yield in vineyards; this translates into $25,000-40,000/hectare left on the table. In addition to being economically important, grapes and wine are a culturally significant crop in human history; having gods dedicated to their existence. The diseases engendered by the grapevine virome do not have clear etiologies as cultivars commonly exhibit complex infection with multiple viral species. To determine disease origin, full-length infectious cDNA clones have been created to singularly infect a virus-free stock of grapevine and observe the resulting physiological and morphological symptoms. This research aims to produce wildtype and GFP-tagged clones for Grapevine rupestris stem pitting-associated virus strain Pinot Noir, as has already been done for strains Syrah and Grande Glabre. Following the successful restriction-based cloning of custom cDNA fragments, *Nicotiana benthamiana*; an herbaceous experimental host, will be used to verify the clone’s ability to infect plant tissues. Subsequently, replication kinetics will be quantified and symptoms observed in the native *Vitis* host. Fluorescence microscopy, Western blotting, and RT-PCR will all be employed to verify the presence, identity, and quantity of the viral clones, respectively. The GRSPaV strains (PN, SY, and GG) would differ in their virulence and replication kinetics in their natural grapevine host. These differences in pathology will allow us to establish disease etiology for rupestris stem pitting disease. Understanding the fundamental causal agents in grapevine viral infections will be crucial to developing effective mitigation strategies to help safeguard viticulture.