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## Environmental (eDNA) detection tools for monitoring Vespa velutina

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The yellow-legged hornet (Vespa velutina) recently invaded the southeastern United States, emerging as a new threat to North American pollinators. In introduced areas, the hornet disrupts plant-pollinator networks and demonstrates a preference for the western honey bee (Apis mellifera L.) as prey, compromising bee colony health. Due to the difficulties associated with locating V. velutina populations, better tools for rapid, accurate hornet detection and monitoring are urgently needed. Here, we investigated the utility of environmental DNA (eDNA) methods to detect the yellow-legged hornet in Savannah, Georgia, USA. We targeted: 1) sentinel apiaries within the known range of the hornet (sampled monthly) and 2) "rapid response" apiaries where hornets had been observed hawking. We collected eDNA samples by swabbing various apiary-associated substrates including hive lids, entrances, and tools, as well as cement pads beneath hives and nearby hornet bait traps. Soil was also collected from the apiary environment. For all samples, DNA was extracted and screened via quantitative PCR for general invertebrate and V. velutina DNA. We found that our eDNA assay accurately detected hornet DNA from positive control swabs collected from V. velutina nests, yet infrequently detected hornet DNA from apiary materials (overall, less than 5% of samples). We detected hornet DNA most frequently from hornet bait traps with recent adult hornet captures (23% of positive traps). These results match hornet behavior, as adults catch their prey in flight and rarely, if ever, contact hive surfaces. Therefore, current eDNA approaches in apiaries do not seem to be effective at monitoring the invasion of V. velutina. Future work can investigate hornet landing behavior to determine more appropriate substrates for eDNA surveillance. Additionally, alternative methods of monitoring the yellow-legged hornet should be explored, such as harmonic radar tracking and camera traps.