Scientific Note

Evaluation of a commercially available ELISA kit for quantifying imidacloprid residues in *Erythrina sandwicensis* leaves for management of the *Erythrina* gall wasp, *Quadrastichus erythrinae* Kim

The *erythrina* gall wasp (EGW), *Quadrastichus erythrinae* Kim 2004, was first detected in Hawaii in 2005 and has been infesting and killing *Erythrina* trees throughout the island chain since. It is believed EGW originated from Africa (Messing et al. 2009). Its host range appears to be limited to *Erythrina*; its geographic range already includes much of Asia and the Pacific. In North America, EGW has recently become established in south Florida and it is expected that introductions will occur to southern California (Smith et al. 2007). Observations indicate that a highly favored host is *E. variegata* L., but numerous species, including the Hawaiian endemic, *E. sandwicensis* O. Deg are severely injured and killed. *Erythrina* are warm-loving plants with about 115 species in the genus. It is expected that EGW will expand its geographic range to meet that of its host *Erythrina* with some restrictions due to climate (Li et al. 2006).

Successful management of newly introduced pests is most probably achieved when multiple approaches are considered (e.g., Hain 2006). Hawaii has a major effort underway in classical biological control and it is hoped that introduced natural enemies will provide a long-term management solution for EGW populations (Hawaii Department of Agriculture 2008). The vagaries of prescribed introductions, along with environmental heterogeneity, differing resource values, cultural and social impacts, and potential effects on nontarget organisms (including humans), all suggest there is a need for the development of multiple tools for management. Indeed, insecticides will continue to play an important role against newly introduced alien species and for protecting particularly valuable resources. For EGW, the active ingredient imidacloprid has been demonstrated to have good activity. Systemic applications of imidacloprid have been effective in killing EGW larvae, and imidacloprid residues in host leaves have been correlated with EGW mortality (Xu et al. 2006, 2008). Applications by stem-injection or soil drench are seen as being valuable for protecting or perhaps rehabilitating high-value *Erythrina* trees, especially ornamentals. In addition, strategic applications of systemics could help reduce new establishments, for example, by treating trees near ports considered likely for introductions. The ability to simply and reliably determine residual concentrations of imidacloprid in *Erythrina* tissues is important for efficient insecticide use, development of effective management plans, and regulatory oversight.

Residual concentrations of imidacloprid in plant tissues are typically determined by high performance liquid chromatography (HPLC). This technique is limited by incomplete resolution of metabolites and relatively high detection limits when coupled with conventional detectors. The other principal approach for determining insecticide residues, gas chromatography (GC), cannot be used for imidacloprid unless it is derivatized to volatile compounds beforehand. These techniques require labor-intensive and time consuming sample preparation steps. Analyses by HPLC/MS/MS techniques are also common, and provide highly selective, quantitative
results. However, they are expensive, requiring specialized laboratory equipment and highly skilled personnel. Determination of insecticide residues by enzyme-linked immunosorbent assay (ELISA) has been used since the late 1980s (Kaufman & Clower 1991, 1995; Van Emon & Lopez-Avila 1992). This technique has the primary advantage of being less expensive under many conditions, but it is considered semi-quantitative by the US-EPA because results are based on indirect competitive immunological responses and not on fundamental properties of the analyte itself, e.g., ultraviolet absorbance or molecular mass (Van Emon 2001). Both HPLC and ELISA require that each particular sample matrix of interest be evaluated for interferences. With ELISA it is common to observe matrix interferences prior to significant sample dilutions (e.g., Byrne et al. 2005).

Most commercial immunoassays are "polyclonal"; that is, they are based on a cluster of antibody strains raised against the target analyte. These commonly exhibit a spectrum of cross-reactivities against not only the target, but also against similar molecules and metabolites. This reduces their selectivity and sensitivity in complex biological matrices like fruits, vegetables and leaf tissues. Further refinement can be obtained by the isolation and testing of individual antibody strains to locate a single one (a monoclonal) with the least cross-reactivity in a particular matrix. Few of these "monoclonal" assays have been commercialized. Xu et al. (2006) applied a monoclonal antibody, which they developed, to imidacloprid residues in E. sandwicensis and found excellent agreement with HPLC results. However, their antibodies are not commercially available and they do not address the utility of commercial ELISA kits for use with Erythrina.

Commercially available ELISA kits are regularly used for detection and semi-quantification of imidacloprid residues in plant tissues as they relate to target insects and the development of integrated pest management (IPM) programs (e.g., Cowles et al. 2006, Byrne et al. 2005). Use of the polyclonal antibody based kit produced by EnviroLogix™ (Portland, ME, USA) has been successfully applied with plant tissues following systemic applications of imidacloprid (Byrne et al. 2005, Cowles et al. 2006). However, the kit is known to provide erratic results (including false-positives) when evaluations are completed for trace concentrations (<1 ppb) in complex matrices (Byrne et al. 2005; Fischer & Michael unpublished data). Sample dilutions typically resolve this issue at higher analyte concentrations (e.g., Byrne et al. 2005). Because imidacloprid concentrations that are realistic for insect control usually also require that samples be diluted (to meet the linear range of the kit), the polyclonal kit has proven sufficient for many entomological applications.

Xu et al. (2006) determined that systemic residues of about 8 ppm imidacloprid in Erythrina leaf tissues were necessary to reduce EGW emergence by about 80%. They observed residue concentrations in E. sandwicensis leaf tissues from 0 to about 8 ppm. Concentrations of interest to managers that target EGW are in that range or above, implying that the commercially available ELISA kit may be adequate for applications with EGW.

The objective of this study was to evaluate the suitability of the commercially available EnviroLogix ELISA kit for determining imidacloprid concentrations in E. sandwicensis leaf tissues. The kit is relatively inexpensive (about $9.00 per sample when run in duplicate as per kit instructions) and simple to use. For confirmation, we adapted existing HPLC methods (Baskaran et al. 1997) and then compared the concentrations determined from this method to those obtained using the EnviroLogix polyclonal kit.
(Figure 1). Using dried *E. sandwicensis* leaves as the matrix, methods were developed and then directly compared on common samples. However, the limited dynamic range of this ELISA kit (0.2–6.0 ppb) necessitated additional dilutions and repeated analyses for samples with higher concentrations of imidacloprid.

Naturally growing *Erythrina sandwicensis* at Pu‘u-O-Kali, Maui, Hawaii were injected with a 5% imidacloprid formulation (IMA-jet, Arborjet, Inc. Woburn, MA, USA) in January 2007 (see Doccola et al. 2009 for treatment and site details). Leaves were collected for determination of insecticide residues at 35 days and 13 months following injections. Upon collection, leaf samples were frozen (−19°C) until processing. For processing, frozen leaves were lyophilized under vacuum for 2 days, ground in a Wiley mill and again frozen. For extraction, dried tissues were allowed to warm to room temperature and 1 g samples were weighed into 15 mL glass vials. Each sample was extracted with 10 mL acetonitrile, agitating on a table shaker overnight. Solids were allowed to settle out and 1 mL of supernatant was withdrawn with a glass pipet and transferred to a 16 × 100 mm culture tube. Acetonitrile was evaporated under a gentle stream of high-purity nitrogen on a 35°C water bath for about 30 min. The residue was redissolved in 1 mL methanol, agitating as necessary, then diluted with 9 mL of deionized water and stored in a refrigerator at 4°C until analysis.

Analysis by HPLC was performed using a Phenomenex Luna C8(2) column, 5 um, 4.6 × 150 mm, eluted with 18/82 (v/v) acetonitrile/water at 1.0 mL/min at 40°C. Detection was by UV absorbance at 270 nm. The minimum detection limit using this method was determined to be 1 ng/mL of leaf extract, or 0.1 μg/g of dry leaves.

Polyclonal ELISA was performed using Quantiplate Kits for imidacloprid (EnviroLogix, Portland, Maine). Plate absorbance values were read at 450 nm.
using a Biotek ELx808 plate reader (Biotek, Inc., Winooski, Vermont) and results calculated using 4-parameter curve fitting software (Biotek Gen5). Any samples outside the kit’s specified calibration range (>6.0 ppb) were diluted 30x with deionized water and reanalyzed. All kit instructions were followed, including analyzing each sample in duplicate, the mean of which is reported.

Results obtained with the commercially available polyclonal ELISA kit compared favorably with those from HPLC, indicating that the kit is adequate for quantifying imidacloprid residues in E. sandwicensis leaf tissue matrices at concentrations relevant for EGW management (Figure 1). Linear regression was used to compare results obtained with the Quantiplate kit with those from HPLC on 83 common samples. The resulting regression equation was highly significant with an excellent fit ($P < 0.0001$; $y = 1.2461(x) + 1.662$ and $r^2 = 0.95$; Figure 1). The slope is greater than 1 indicating that, on average, the ELISA results are greater than those obtained by HPLC by about 25%. This is probably because the polyclonal ELISA antibodies detect imidacloprid metabolites along with imidacloprid (EnviroLogix 2006). If desired, ELISA readings could be “corrected” using a regression equation like that in Fig. 1; however, additional validation and appropriate caution should be employed if such a procedure is pursued.

Because 8 ppm imidacloprid in leaf tissue has been identified as the concentration at which ~80% of EGW are killed (Xu et al. 2006), this (or another) value could be used in the future to indicate whether tissues were effectively protected. Using a 30x extract dilution, the kit response range of 0.2 to 6.0 ng/mL corresponds to a dry leaf concentration range of 0.6 to 18 μg/g (ppm), bracketing the minimum control level for EGW. Good quantitative results can be achieved in this range and, so long as quantitation is not desired for concentrations above 18 μg/g, additional dilutions would be unnecessary and this approach would provide a relatively inexpensive method to refine IPM programs and evaluate applications (e.g., for regulators). In our evaluation, 94% of samples (78 of 83) would have been categorized correctly by the ELISA kit using the 8 ppm threshold provided by Xu et al. (2006) and the HPLC result as the standard. In each incorrect case, the ELISA result was higher, something that may be improved upon in the future (e.g., if the Xu et al. monoclonals become available) but a good result nonetheless. The polyclonal EnviroLogix ELISA kit appears adequate for determining imidacloprid residues in Erythrina leaf tissues as they relate to EGW management applications, and offers a commercially available and relatively inexpensive option for these determinations.

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