Insecticidal resistance is becoming increasingly problematic in commercially important pest species and insect cytochrome P450s have been implicated in conferring resistance by metabolizing and/or inactivating compounds.\(^1\) A means of determining whether novel compounds interact with the cytochrome P450s thought to be involved in resistance would, therefore, be a useful tool to look for resistance liabilities in compounds early in their development. Here we have used three CYP isoforms from two pest species, CYP6CM1 (B. tabaci) and CYP392A11 and CYP392E10 (T. urticae) to set up a simple screen for inhibition of enzyme activity using fluorogenic substrates. The CYPs were coexpressed with NADPH P450 reductase (from A. gambiae with CYP6CM1 or from T. urticae with CYP392A11 and CYP392E10) in E. coli. Bacterial membranes containing CYPs were incubated with fluorogenic substrates in 50 mM potassium phosphate pH 7.4, 5 mM MgCl\(_2\) at 37°C. The reaction was started by the addition of an NADPH generating system and fluorescence was monitored continuously over 30 minutes. CYP inhibition was determined using a 3-fold serial dilution (from 50 µM to 0.07 µM final concentration) of inhibitor down a 96 well plate. The reaction was started by the addition of an NADPH generating system and fluorescence was monitored continuously over 30 minutes.

### Methods

Recombinant CYP6CM1, CYP392A11 and CYP392E10 were co-expressed with mosquito (CYP6CM1) or urticaria (CYP392A11 and CYP392E10) NADPH P450 reductase in E. coli in 10 litre fermenters. Mosquito cytochrome P450 was expressed in E. coli and partially purified over a Ni-agarose column. Expression constructs for the insect CYPs were kindly provided by Prof. J. Vontas, Associate Professor Biotechnology & Applied Biology, University of Crete. Bacterial membranes containing CYPs were incubated with fluorogenic substrates in 50 mM potassium phosphate pH 7.4, 5 mM MgCl\(_2\) at 37°C. The reaction was started by the addition of an NADPH generating system and fluorescence was monitored continuously over 30 minutes. CYP inhibition was determined using a 3-fold serial dilution (from 50 µM to 0.07 µM final concentration) of inhibitor down a 96 well plate. The insecticide stock solutions (5 mM) were prepared in DMSO (present in the assay at 1.5% or 2.5% [v/v]). Wavelengths for detection of the compound increased with time implying that it may be a substrate for the enzymes and thus would be broken down as a result of metabolism by the CYP. In other cases, e.g. CYP6CM1 with malathion, the IC \(_50\) falls with time indicating that time is a result of metabolism by the CYP. In other cases, e.g. CYP6CM1 with malathion, the IC \(_50\) falls with time indicating that time

### Results

Initial incubation of CYPs with the six substrates listed in Table 1 highlighted DEF as the most promising substrate to use in an assay. CYP6CM1 also showed activity with 7-ER, CEC and CYP392A11 and CYP392E10 were only active with DEF. The activity of CYP6CM1 and CYP392E10 increased 2.5 and 10 fold respectively with the inclusion of b5 cytochrome P450.

### Conclusion

We have used recombinant insect pest CYPs that have been implicated in conferring resistance to set up a rapid screen for their inhibition and to look for resistance liabilities in compounds early in their development. The results obtained suggest that some compounds are not substrates for the CYPs, whereas others are substrates and would be broken down as a result of metabolism by the CYP. In other cases, e.g. CYP6CM1 with malathion, the IC50 falls with time indicating that time

### References