

DNA-based Genotyping Techniques for the Detection of Point Mutations Associated with Insecticide Resistance in Colorado Potato Beetle (CPB)

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Three DNA-based genotyping techniques, including bi-directional PCR amplification of specific allele (bi-PASA), single stranded conformational polymorphism (SSCP), and minisequencing, have been developed and compared for the detection two point mutations associated with insecticide resistance in the Colorado potato beetle (CPB). Bi-PASA, employing two allele-specific primers, was determined to be the most efficient and rapid genotyping method for the simultaneous detection of both resistant/susceptible homozygous and heterozygous alleles. Its resolution, however, was strongly dependent on the quality of template genomic DNA. SSCP also allowed clear genotyping including the detection of heterozygous allele and was less dependent on template DNA quality but required a longer time commitment. Minisequencing was amenable to a 96-well microtiter plate format for the processing of a large number of samples and allowed direct detection of resistant/susceptible homozygous alleles but was not as efficient as the PASA and SSCP in detecting heterozygous allele. In conclusion, considering the advantages and disadvantages of each technique, DNA-based genotyping is best employed in combinations, with the bi-PASA as the primary method and the SSCP and minisequencing as the secondary validating methods. Availability of these DNA-based genotyping techniques using neonate genomic DNA as rugged, rapid, and cost-effective alternatives for the traditional bioassay will allow the precise monitoring of the resistant and susceptible allele frequencies, including heterozygous type, in the field populations of CPB.