DEVELOPMENT OF GENETICALLY ENGINEERED WALNUTS FOR CONTROL OF STORED-PRODUCT AND QUARANTINE PESTS

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Bacillus thuringiensis Berliner (Bt) produces insecticidal activity by means of a crystalline inclusion body. These inclusion bodies contain a protein referred to as an insecticidal crystal protein fragment (ICPF). ICPFs are considered safe biological control agents as they have no known direct effect(s) on non-target organisms. Products containing Bt have been marketed for many years as biological control agents under a number of trade names. Recently several genes encoding ICPFs effective against lepidopteran insects have been isolated from different strains of Bt and their DNA sequences determined. Insertion in plant genomes of these sequences have led to the development of transgenic tobacco (Vaeck *et al.* 1987), tomato (Fischoff *et al.* 1987), and cotton plants (Perlak *et al.* 1990). Reductions of pink bollworm and other lepidopterous insect damage to transgenic cotton plants (Bollgard™) containing an ICPF have been reported. Little or no effect on beneficial insects have been noted in studies with these plants.

The toxicity of two ICPFs *cry*IA(b) and *cry*IA(c), encoded by *cry*IA(b) and *cry*IA(c) genes from the Bt strains HD-1 and HD-73, respectively, have been reported. Both protein fragments were toxic to larvae of the codling moth, *Cydia pomonella* (L.), navel orangeworm, *Ameyelois transitella* (Walker), and Indianmeal moth, *Plodia interpunctella* (Hübner). Indianmeal moth larvae, a storage pest, were most susceptible; navel orangeworm the least susceptible of the three species. Codling moth, a quarantine pest, was intermediate in susceptibility. Dandekar *et al.* (1994) demonstrated that gene(s) responsible for the production of ICPFs had been incorporated into the walnut genome. However, levels of expression of ICPFs were low and provided little or no insecticidal activity to the three pests of concern (Dandekar *et al.*, 1994).

"Second generation" engineered walnuts were described and produced by A. M. Dandekar and G. H. McGranahan of the Department of Pomology, University of California, Davis. Somatic embryos of a repetitively embryonic cell line designated SU2, originally described from an open pollinated seed of the *Juglans regia* L., cultivar 'Sunland', were used for transformations (Tulecke and McGranahan 1985). A synthetic full-length gene (*cry*IA(c)) was supplied by Dr. D. Fischhoff of Monsanto Co., St Louis, Missouri. A large number of embryos were transformed according to the methods

of Dandekar *et al.* (1994) that expressed the synthetic gene *cryIA(c)* using the CaMV35S regulatory sequences. Presumptive evidence of transformation was based on marker genes for kanamycin resistance and GUS, and southern blot analysis. Putative embryos were weighed, lyophilized, weighed again, and assayed for insecticidal activity. Plantlets originating from some of these embryos were also lyophilized and assayed as whole plants or plant parts. We assayed 61 putative embryos and categorized (high, moderate and low activity) them based on the level of insecticidal activity and/or their effect on larval development after 7 days of feeding. One replication consisted of 10 larvae per embryo line; 2 or 3 replications were run per line. Twenty-one high expression lines expressed ICPFs at levels sufficient to kill most or all of the test insects and completely stopped larvae development, e.g., all had died or were still first instar after 7 days. A moderate level of expression was shown in 12 of the transformed lines. The remaining 28 lines expressed low levels of ICPFs.

The studies show that high levels of ICPFs can be produced in walnut. About one-third of the embryos tested completely arrested larval development and caused 90 to 100% mortality. Plantlets originating from high expressing embryos also showed high levels of expression either intact or when separated into leaflet, stem or callus portions. The level of control of these production, quarantine and stored-product pests in commercial walnut (vegetative or reproductive tissue) is yet to be determined in the field.

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